In this chapter, emphasis has been placed on the core mechanisms underlying the broad categories of hypersensitivity responses distinguished on the basis of the Gell and Coombs classification and based on differences in the immune reactants (antibodies or cells), the form of the presented antigen, and the effector mechanisms involved. Mechanisms involved in individual drug hypersensitivities including responses to reactive metabolites from chemically “inert” parent drugs such as sulfamethoxazole; relationships between chemical structures and immune responses seen with, for example, anaphylactic reactions to neuromuscular blocking drugs (NMBDs) during anesthesia; hypersensitivities and other intolerances to non-steroidal anti-inflammatory drugs; and mechanisms underlying the killing of malignant cells by some drugs used in chemotherapy are not confined to this chapter but presented in the relevant chapters dealing with pharmacologically different groups of drugs. Most hypersensitivities to drugs manifest as type I or type IV reactions. Type II and type III drug hypersensitive reactions are far less often seen and are considered after the discussions of the types I and IV responses. Mechanisms, to the extent that they are currently understood, of other types of “hypersensitivity” reactions or intolerances, some mediated by antibodies other than IgE and others by cells, are also discussed. We begin by examining the mechanisms underlying type I drug-induced IgE antibody allergic sensitization, regulation, and production and the effector mechanisms operative in IgE-mediated allergic reactions.

3.1 Allergic Sensitization to Drugs and the Dogma of Previous Exposure

3.1.1 Immunogenicity of Free and Conjugated Drugs

As well as the chemical nature of a drug, its size and complexity influence its antigenicity. Chemicals of molecular mass less than 5 kDa and sometimes up to about 10 kDa are often poorly or non-antigenic. From the time of the early immunochernical studies on antigenicity and haptens, organic chemicals of small molecular mass have been assumed to be antigenic and capable of stimulating an immune response only as a complex with a macromolecular carrier, usually protein. By coupling a wide range of different chemicals that are not antigenic in their free state, for example, steroids, sugars, purines, pyrimidines, nucleosides, and aromatic ring compounds such as phenols, etc., Landsteiner and other early investigators demonstrated clear and specific antibody responses in laboratory animals. Chemicals such as drugs may form hapten-carrier complexes in vivo in three different ways – by direct chemical covalent interaction with a soluble or cell-bound protein, by biotransformation of the drug to form a reactive...
metabolite able to bind to a carrier protein, or by degradative changes to the parent molecule forming reactive groupings (Fig. 3.1). In practice, however, it is often not possible to show protein binding by a drug or to even offer a satisfying explanation of how such binding might occur given the known chemical properties of the drug and the metabolic processes to which it is exposed. While a number of allergenic drugs such as the β-lactams undergo well-known ring opening and subsequent protein-binding reactions (Chap. 5), for many drugs, chemical reactivity, protein binding, biotransformation, or the involvement of degradative products and/or reactive impurities has not been demonstrated. This raises the question of another possible mechanism(s) to explain the immune recognition of “small” drug molecules and the subsequent immunological steps involved in the drug-induced hypersensitivity responses (see Chap. 3, Sects. 3.1.2 and 3.1.3, Chap. 6, Sect. 6.3.3.3, and Chap. 7, Sects. 7.2.2.3, 7.2.5.3). Over the last few decades, many drugs have been implicated as provoking agents for a range of hypersensitivity states, and although the list of identified drug allergenic determinants has expanded, this aspect of hypersensitivity research is still in its infancy. Structures as diverse as substituted ammonium ions, simple disaccharides, small side chain groups and ring structures on β-lactams, halogenated isoxazolyl groups, and whole molecules as seen with trimethoprim and chlorhexidine are known to be recognized as allergenic determinants in some drug-allergic patients. Of the presently known drug allergenic determinants, most have been reliably identified in IgE antibody recognition studies using quantitative immunochromatographic hapten inhibition techniques. The approaches and methods already applied so successfully to a range of drugs (see Chaps. 5, 6, 7 and 8) need to be expanded to cover other yet-to-be-defined IgE antibody-binding determinants and extended to T cell-mediated drug hypersensitivity reactions where progress has been slow. Results from the IgE studies have demonstrated that more than one allergenic determinant generally occur on drugs and such antigenic heterogeneity is reflected in patients’ IgE antibody recognition responses. As more drug allergies and more allergic individuals are studied, the extent of this heterogeneity will emerge and with it the possibility of gaining greater insights into the structural basis of drug allergenicity.

For an allergic reaction to a given drug, immunological dogma requires that the response occurs on re-exposure to the drug after the initial sensitizing exposure to that drug. However, this seemingly obvious requirement may not always hold true or appear to hold true. Some allergic responses, sometimes even life-threatening as with anaphylaxis, occur on first exposure to a

**Fig. 3.1** Diagrammatic representation of possible, and potentially allergenic, hapten-protein complexes that may form in vivo from a drug and/or its metabolite(s) and degradative product(s). (Reproduced with permission from Baldo BA, Pham NH. Structure-activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol. 1994;7:703–21. Copyright 1994 American Chemical Society)
drug. Such reactions to the NMBDs are well-known, and there are numerous other investigations and case studies involving a variety of pharmacologically different drugs including trimethoprim, some iodinated contrast media, opioids, and some antibiotics that report the same phenomenon. In some cases, this might be explained by previous exposure to a structurally similar drug or to a structurally similar compound that may not even be administered as a drug. An example of the former case is a reaction to a cephalosporin in a patient previously given a penicillin, while a reaction to a drug may also result from previous exposure to the drug (e.g., an antibiotic in meat) or an antigenically cross-reactive chemical in some foods or in the environment. Although IgE antibodies are almost invariably thought of as induced humoral responses to allergens, parasites, and fungi, some of the antibodies are “natural,” that is, antibodies formed without exposure to foreign antigens via infection or passive or active immunization. Examples of such antibodies appear to be those that are complementary to various cross-reactive carbohydrate determinants (the so-called CCDs) and to phosphorylcholine connected by phosphodiester linkages in some N-linked proteoglycans and glycolipids and found in pneumococcal teichoic acid (“C substance”) and other “C substances” in bacteria, fungi, arthropods, helminths, protozoa, and plants. The curious connection between IgE natural antibodies and the alphaL-linked D-galactose disaccharide found on cetuximab, a chimeric mouse-human IgG1 monoclonal antibody used for cancer treatment, and anaphylaxis in some treated patients and the possible cross-reaction of natural anti-phosphorylcholine IgE antibodies with ammonium groups on NMBDs (Chap. 7, Sect. 7.2.2.1) occur in many drugs and chemicals frequently encountered by humans. Bioisosteres, that is, groups with similar physical and/or chemical properties that impart similar biological properties to a drug, should also be kept in mind when prior allergic sensitization is suspected in patients who are first time reactors to a drug. In this era of so-called rational drug design, bioisosteres are commonly seen, for example, in the replacement of a six-membered phenyl ring with a five-membered thiophene ring in many synthesized drugs. An example of the importance of bioisosterism in the identification of allergenic structures and allergic cross-reactivity is discussed further in Chap. 5, Sect. 5.2.4.2.1.

3.1.2 Mediator Release by Free and Conjugated Drugs in Immediate Allergic Reactions

In allergic subjects, IgE antibodies, as well as being free in serum, are fixed to the extracellular D1 distil and D2 proximal domains of the FcεRI receptor on mast cells and basophils via the Cε2 and Cε3 domains of the antibody Fc region. Bridging of adjacent cell-bound IgE molecules by
at least bivalent allergenic determinants reacting with their complementary antibody combining sites (Fig. 3.2a–d) triggers cell degranulation and release of a variety of mediators that cause the signs and symptoms of a type I hypersensitivity reaction. In the case of drug-carrier conjugates, cross-linking of IgE antibodies is readily explained by the presence on the conjugates of multiple reactive drug determinant sites, but for free, uncomplexed drug molecules, both the size and number of reactive determinants would appear to be too small for cross-linkage of antibody combining sites to occur. Drugs with a single IgE-binding determinant cannot, of course, cross-link adjacent cell-bound antibody molecules (Fig. 3.2e), but even if two or more determinants are present, they must be separated by a suitable distance and/or be suitably spatially arranged if cross-linking via adjacent complementary antibody combining sites is to occur (Fig. 3.2f, g). Despite this problem of explaining the mechanism of apparently monovalent drug-induced allergic mediator release, there is at least one group of drugs, the neuromuscular blockers (and probably more to be identified), that can specifically elicit antibody-induced mast cell activation and release without first undergoing coupling to a macromolecular carrier. For these drugs, di- or multivalency is an inherent part of the molecular structure, and, even in the absence of protein binding, cross-linking of cell-bound antibodies can be effected (Fig. 3.2a). Of the polymethylene bismethonium compounds, the 2 nm molecular length of the C-10 congener decamethonium is optimal for neuromuscular block, that is, it best fits the distance between the receptive sites on the muscle nicotinic acetylcholine recep-

**Fig. 3.2** Different ways in which a free drug (shown in bold in a, b, e, f, and g) and a drug-protein conjugate (c, d) may cross-link or bridge adjacent cell-bound IgE molecules which triggers release of the mediators of immediate hypersensitivity. (a) Bridging via an allergenically divalent unconjugated drug molecule with the same or closely related allergenic determinants. This is the mechanism thought to occur in patients who experience anaphylaxis following administration of a neuromuscular blocking drug. (b) Bridging via a free, unconjugated drug molecule containing two (or more) different determinants that elicit an IgE response. (c, d) Bridging via conjugated drug molecules with cross-linking effected by the same, or different, determinants, respectively. Failure to bridge adjacent cell-bound IgE molecules because. (e) drug is allergenically monovalent; (f, g) drug determinants are not positioned to effect cross-linkage. (Adapted with permission from Baldo BA, Pham NH. Structure-activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol. 1994;7:703–21. Copyright 1994 American Chemical Society)
Interonium distances, however, are less, for example, 1.4 nm for decamethonium and succinylcholine (which, however, has a bent conformation), 1.08 nm for \( d \)-tubocurarine (molecular length 1.8 nm), and 1.14 nm for pancuronium (molecular length 1.9 nm). These distances appear to be suitable for the neuromuscular blockers to bridge and thus activate adjacent IgE molecules on the mast cell surface (see also Chap. 7, Sects. 7.2.2.1 and 7.2.2.3).

### 3.1.3 Immunological Recognition of Free, Unconjugated Drug Molecules

The generally accepted explanation for the recognition of drugs causing an immune-mediated hypersensitivity reaction is based on the binding of drug to a protein carrier molecule, immune recognition and processing of the drug-protein complex, presentation of drug-peptide conjugates to the T cells, and recognition and reaction of the T cell with the drug antigen. However, although there is no evidence that many drugs, either as the parent compound or a metabolite, bind to a suitable carrier, there is evidence that T cells recognize metal ions such as Ni\(^{2+}\) and some drugs like sodium aurothiomalate that do not require antigen processing. In one explanation, the drug is said to bind directly to self-peptides in the antigen-binding cleft of the major histocompatibility complex (MHC). In another possible alternative, the drug may couple directly to the MHC itself on regions involved in binding to the T cell receptor. In drug interaction with the MHC, recognition may be restricted to a limited number of peptides, or it may be promiscuous, that is, independent of peptide. For some drugs at least, direct stimulation of T cells via the T cell receptor in an MHC-dependent way has been suggested. With sulfamethoxazole, for example, a drug known to be metabolized to its reactive nitroso derivative, only a minority of T cell clones reactive with this metabolite were isolated from sulfamethoxazole-allergic patients. The short time period for T cell activation to occur with some free, unmetabolized drugs, T cell clone reactivity with glutaraldehyde-fixed antigen-presenting cells, and removal of free drug by washing all suggest a drug–T cell receptor interaction that is independent of metabolism and processing. Further consideration of the recognition and the immune response to free, unconjugated drugs is set out in Sect. 3.9 below.

### 3.2 IgE Antibodies, Mast Cell Receptors, and IgE-Mediated Drug-Induced Reactions

The central importance of IgE antibodies in both the immediate and late phases of an allergic response involving inflammatory reactions is well established. The IgE and IgG molecules show two immediate and obvious differences, firstly in the 6 domains (a dimer of Ce2-Ce3-Ce4) that make up the Fc piece of IgE compared to 4 domains (a dimer of Cy2-Cy3) of the IgG Fc piece and secondly the absence of a hinge joining the IgE H chains (Fig. 3.3a, b). The Ce3 and Ce4 domains of IgE are homologous to the Cy2 and Cy3 domains of IgG suggesting that the hinge region of IgG has replaced the Ce2 domains of IgE. The two extra (Ce4) domains do not impart a more extended Y-shape to the IgE molecule, but a more compact structure with the Ce2 domains bent back over the Ce3-Ce4 region. Despite the absence of a hinge region, the Ce2 domains can twist, extend, and bend relative to the Ce3-Ce4 region, making the IgE molecule highly flexible (Sect. 3.2.2). Glycosylation is essential for functional IgE antibodies. Both IgE and IgG are glycosylated. Asn394 in the Ce3 domain of IgE is fully glycosylated like its homologous site Asn297 in the Cy2 domain of IgG, but the glycosylation at Asn394 is of the high mannose type, whereas Asn297 in IgG is a complex biantennary heptasaccharide core formed from two N-acetyl-D-glucosamine branches (Fig. 3.3c,d).

IgE mediates the allergic inflammatory response by binding to both its high affinity receptor FceRI on mast cells and basophils and to the low affinity IgE receptor FceRII (CD23) to augment humoral and cellular responses. FceRI, a member of the FcγR family of receptors, is expressed on a wide variety of other cell types including antigen-presenting cells, dendritic...
cells, Langerhans cells, macrophages, eosinophils, neutrophils, monocytes, platelets, as well as human tissue cells of the airway smooth muscle and bronchial and intestinal epithelia. FcεRI-dependent IgE antibody-mediated allergic reactions can therefore occur at these local sites. IgE’s second principal receptor, FcεRII or CD23, the so-called “low-affinity” IgE receptor, is a member of the C-type (Ca²⁺-binding) lectin-like domain superfamily (see Sect. 3.2.4).

### 3.2.1 Initiating Events in the Production of IgE Antibody

IgE is produced by plasma cells at the site of an allergic reaction generally in mucosal, cutaneous, and gut lymphoid tissue. IgE antibody production begins with the interaction between antigen-bearing antigen-presenting cells (APC) and lymphocytes. APCs can be dendritic cells, the most important cell in initiating the adaptive immune response, macrophages, and B cells. Naïve T lymphocytes not only need to have antigen presented in a special way; they also require precise signals to become activated. Both of these requirements are fulfilled by the APC firstly via the membrane-associated MHC that interacts with the T cell receptor (TCR) (activation signal 1) and secondly by the provision of co-stimulatory signals in the form of the membrane protein ligand CD80 (B7-1) working in tandem with another membrane ligand CD86 (B7-2) (activation signal 2). These ligands interact with their complementary receptor CD28 constitutively expressed on naïve T cells to allow the cells to undergo clonal expansion (Fig. 3.4). Resting or naïve B cells are also non-dividing, and to undergo clonal expansion and differentiation to effector B cells, that is, to produce allergen-reactive IgE antibodies, B cells also require the

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**Fig. 3.3** Diagrammatic representations and ribbon diagrams portraying the 3-D protein structures of the IgG and IgE antibody molecules comparing the heavy chain constant domains of IgG and IgE and some detail of the glycosylation of the molecules. The IgG Fc piece comprises 4 domains, a dimer of Cγ2-Cγ3 (a); the 6 domain IgE Fc piece is a dimer of Cε2-Cε3-Cε4 (b). While the Cγ2-Cγ3 domains of IgG are homologous to Cε3-Cε4 of IgE, IgG has a hinge attachment instead of the Cε2 dimer of IgE, and IgE has the extra paired Cε4 domains. Both IgE and IgG are glycosylated. The IgG Cγ2 domain has a complex biantennary heptasaccharide core formed from two N-acetyl-D-glucosamine branches covalently attached to Asn297 (c). IgE is glycosylated with a high mannose carbohydrate attached at Asn394 in the Cε3 domain (d). FUC, fucose; GAL, galactose; MAN, mannose; NAG, N-acetylgalactosamine; SIA, sialic acid. (Reproduced from Sutton BJ, Davies AM, Bax HJ, et al. IgE antibodies: from structure to function and clinical translation. Antibodies. 2019:8:19. https://doi.org/10.3390/antib8010019 an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/)
participation of a specific receptor, the B cell receptor or BCR, and co-stimulation from T helper cells. The BCR has immunoglobulin anchored in the cell membrane, and, in concert with the B cell co-receptor complex, it is the interaction between this surface immunoglobulin and its complementary antigen that initiates B cell activation. Upon binding to the antigen, the BCR-antigen complex is internalized within an endosome, processing follows, and the processed antigen is presented back on the surface by MHC type II molecules. If maturation of the B cell to a plasma cell or a memory cell is to continue, interaction with, and co-stimulation by, an activated T helper cell is required. Interaction between the B cell and an activated Th2 cell with the appropriate TCR involves recognition of the MHC-processed antigen by the TCR and co-stimulator CD80/CD86 (B7) signals. Co-stimulation of the B cell that eventually leads to clonal expansion and isotype switching is also enabled through upregulation of the CD40 ligand (CD40L) (Fig. 3.4). If CD40L-CD40 receptor interaction and co-stimulation do not eventuate, B cells undergo apoptosis and are eliminated. Cell proliferation and isotype switching for the synthesis of IgE are aided by the cytokines IL-4 and IL-13 generated by Th2 cells. These two cytokines initiate transcription of germline mRNA for IgE antibodies and are regarded as the first of two signals necessary for class switching from IgM- to IgE-bearing cells. The second signal is delivered by the interaction of CD40L on the T cell surface with its receptor CD40 on the B cell. This interaction results in all the elements necessary for the ε-heavy chain being brought into close proximity.

IgE antibody levels influence IgE receptor density on cells. High levels of antibody increase both the number of FceRI receptors and the
3.2.2 FcεRI and Allergic Release of Mediators of Hypersensitivity from Mast Cells

The critical role of IgE in both the immediate and late phases of the allergic response is well established, and, together with the mast cell, the resultant humoral and cellular interactions produce the inflammatory mediators and symptoms characteristic of allergic reactions. On the basis of the type of proteases and proteoglycans in their granules, human mast cells can be divided into three populations: tryptase-only positive mast cells in the lungs and intestinal mucosa; tryptase, chymase, and carboxypeptidase positive mast cells in the skin, connective tissues, and intestinal mucosa; and a smaller population of chymase-only positive cells in the nasal and intestinal mucosae. For more details of tryptase and its importance as a diagnostic marker for anaphylaxis, the reader is referred to Chap. 4, Sect. 4.5.1. The initial event in the activation of mast cells for mediator release is the binding of IgE antibodies to the high affinity FcεRI IgE receptor abundantly expressed on the mast cell and basophil surfaces. The high affinity of the receptor (K_a 10^10 M^-1) means that a high proportion of IgE is bound even in situations where there are low levels of circulating IgE antibodies. The FcεRI complex is a receptor in tetramer form made up of a ligand-binding α chain structurally related to the α chains of FcγR, a tr raspan β chain, and the FcγR γ chain dimer (Figs. 3.5 and 3.6a) The α chain has two protruding Ig type domains that bind the Cε3 region of IgE (Fig. 3.6b), and in the presence of the antibody, the receptor is upregulated, while the Fc receptor for IgG is downregulated. The β and γ chains each contain an ITAM (immunoreceptor tyrosine-based activation motif) that interact with the Lyn, Syk, and Fyn protein tyrosine kinases. The critical initial event for mediator release, as occurs in anaphylaxis, is the cross-linking by allergen molecules of IgE antibodies bound to the α chain of FcεRI (Fig. 3.7). The capacity of the Cε2 domains of IgE to twist and bend makes the IgE molecule highly flexible for antigen binding and cross-linking of receptors (Fig. 3.6b). The IgE-FcεRI complex is long-lasting and dissociates exceptionally slowly. Cross-linking of receptors causes their aggregation, rapid migration to lipid rafts, activation of the Lyn and Fyn protein tyrosine kinases, and ulti-
mately transphosphorylation of the β and γ chains and involvement of the Syk kinase. Mast cell degranulation (Fig. 3.8), which can occur within seconds, follows a series of activation steps induced by phosphorylation reactions discussed in more detail in Sect. 3.4.2.

Some of the released mediators of inflammation and anaphylaxis are stored in the cytoplasmic granules including the biogenic amines histamine (see below, Sect. 3.3.1) and serotonin; proteoglycans heparin and chondroitin sulfate; serine and other proteases, mainly tryptase, chymase, cathepsin-G, and carboxypeptidase; lysosomal enzymes β-glucuronidase and β-hexosaminidase; some cytokines including TNF, IL-4, stem cell factor, and fibroblast growth factor; and eosinophil, neutrophil, and monocyte chemotactic factors. The preformed mediators are responsible for the immediate signs and symptoms of vasodilation, edema, bronchoconstriction, and itching. The newly synthesized group of released mediators includes prostaglandin D_2 (PGD_2); thromboxanes; leukotrienes LTB_4, LTC_4, and LTD_4 (Sect. 3.3.2); and platelet activating factor (PAF) (Sect. 3.3.3).

A host of other cytokines (pro- and anti-inflammatory), chemokines, and chemotactic, stimulating, and growth factors including inter-
Fig. 3.6 (a) Diagrammatic representation of the FceRI receptor, a tetramer of two extracellular Ig-like α-chain domains (purple) that bind the Ce3 region of IgE, a tetraspan β-chain and a γ-chain dimer. The β- and γ-chains each contain ITAM signaling motifs (purple). (b) Crystal structure of IgE Fc complexed with FceRIα showing the compact conformation with the Ce2 domains bent back toward the Ce3 and Ce4 domains. (Reproduced with permission from Sutton BJ, Davies AM. Structure and dynamics of IgE–receptor interactions: FceRI and CD23/FceRII. Immunol Rev. 2015;268:222–35 and Sutton BJ, Davies AM, Bax HJ, et al. IgE antibodies: from structure to function and clinical translation. Antibodies. 2019;8:19. https://doi.org/10.3390/antib8010019 an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/))

Fig. 3.7 Diagrammatic representation of cross-linking by allergen (green) to the combining sites of two identical FceRI receptor-bound IgE antibodies bound to the extracellular α1 and α2 domains of FceRI (purple). (Reproduced from Sutton BJ, Davies AM, Bax HJ, et al. IgE antibodies: from structure to function and clinical translation. Antibodies. 2019;8:19. https://doi.org/10.3390/antib8010019 an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/))
3.2 IgE Antibodies, Mast Cell Receptors, and IgE-Mediated Drug-Induced Reactions

**Fig. 3.8** Diagrammatic representation of antigen-induced degranulation of, and mediator release from, mast cells by antigen-effected cross-linking of adjacent cell-bound complementary IgE antibodies.

- **Resting mast cell containing granules and inflammatory mediators**
- **Activated mast cell**
- **IgE antibody**
- **electron dense granules**
- **FcεRI**
- **Antigen cross-links adjacent antibodies**
- **granules (less electron dense)-**
  - contents released & open to exterior
- **Mediators of hypersensitivity and inflammation**
  - Contracts airways smooth muscle
  - Stimulates secretions of mucous glands
  - Dilates and increases permeability of blood vessels
  - Activates platelets
  - Attracts eosinophils
leukins −1, −3, −5, −6, −8, −9, −10, −11, and −13, GM-CSF (granulocyte-macrophage colony-stimulating factor), MCP-1 (monocyte chemotactic protein-1), RANTES (regulated on activation, normal T cell expressed and secreted; CCL5), and CCL-11 (eotaxin) are also released.

The central role of FcεRI in IgE-mediated allergic release of inflammatory mediators, cytokines, and enzymes leading to the symptoms of an immediate allergic reaction has recently been exploited in experiments that may prove to be the forerunner for effective and rapid desensitization and prevention of such reactions, particularly anaphylaxis. Following the recent demonstration that IgE-mediated anaphylaxis in wild-type BALB/c mice can be prevented by immunization with a monoclonal anti-mouse FcεRIα, FD Finkelman and colleagues extended the approach to humans by passively immunizing humanized (huFcεRIα/F709) mice and reconstituted immune deficient (reNSGS) mice with anti-human FcεRI monoclonal antibodies. The antibodies effected rapid desensitization of mice sensitized with egg antigen or by intravenous injection of anti-huIgE monoclonal antibody. Anaphylaxis was suppressed more rapidly than seen with the monoclonal antibodies omalizumab or ligelizumab, both of which target the Igε chain C region (Sects. 3.5.2 and 3.5.5). As well as suppressing IgE antibody-mediated anaphylaxis, the anti-human FcεRI monoclonal antibodies removed >98% of the IgE antibodies from mouse peritoneal mast cells. The authors concluded that “rapid desensitization with anti-human FcεRI monoclonal antibodies may be a safe, effective, and practical way to prevent IgE-mediated anaphylaxis.”

3.2.3 Amplification of IgE Antibody Production by Cellular Interaction

Mast cells, basophils, and even dendritic cells can accentuate B cell production of IgE antibodies by direct interaction (Fig. 3.9). IgE antibodies newly synthesized by plasma cells bind to the FcεRI receptors on the surfaces of mast cells and basophils. Cross-linkage of antibodies by antigen that reacts with the combining sites of adjacent receptor-bound IgE molecules activates the receptors and triggers the cells to express CD40 receptors on the B cell surface inducing class switching and the production of IgE antibodies.

![Fig. 3.9 Amplification of IgE antibody production by B cells by direct interaction of mast cells expressing CD40L and secreting IL-4. These interact with their complementatory receptors on the B cell surface inducing class switching and the production of IgE antibodies.](image)
ligand (CD40L) and secrete IL-4. These molecules react with their complementary receptors expressed on the B cell surface, and hence, like Th2 cells, mast cells and basophils can induce class switching and increase the production of IgE antibody.

3.2.4 Low Affinity IgE Receptor FcεRII (CD23)

A second receptor for IgE, the low affinity receptor FcεRII also known as CD23 is expressed on several types of hematopoietic cells including mature B lymphocytes, T cells, macrophages, monocytes, dendritic cells, and eosinophils, as well as airway smooth muscle and gut epithelial cells. The receptor consists of triple α-helical coiled chains with C-terminal tails supporting three identical C-type lectin-like domain that binds IgE (Fig. 3.10). The designation “low” affinity is derived from the receptor’s lower affinity (K_D for each of its lectin-like heads ~10^6 M⁻¹) than the affinity of the FcεRI receptor (K_D = ~10^{10}–10^{11} M⁻¹). CD23 has multiple functions by virtue of its capacity to bind a range of different ligands. As well as binding IgE in both its secreted and B cell-bounded form, CD23 binds CD21 (also known as complement receptor 2), CD18/CD11b (complement receptor 3), CD18/11c (complement receptor 4), and α_β₃, the vitronectin receptor. CD23 is a 45 kD type II membrane protein with homology to calcium-dependent (C-type) lectins. It is involved in both the up- and downregulation of IgE synthesis by B cells, augmentation of humoral and cellular responses, and facilitation of the phagocytosis of IgE opsonized antigens. Upon antigen-mediated cross-linking of bound IgE, the low affinity receptor on B cells downregulates IgE synthesis. Augmentation of IgE-mediated responses can be demonstrated in vivo by the prevention of an immunogen and antigen-specific IgE-induced increase in serum IgE titers following pretreatment with anti-CD23 antibodies. As well as its effects on the FcεRI receptor, IgE can also upregulate CD23 resulting in an increased allergic response in the bronchial mucosa. This is thought to occur via enhancement of allergen uptake and presentation. Considering the effects of IgE on the high and low IgE receptors, it seems that inhibition of the antibody leads to downregulation of both receptors and ultimately decreased mediator release from mast cells and basophils.
Important findings on CD23 control of IgE antibody synthesis and homeostasis in human B cells have recently been forthcoming. The endogenous metalloprotease, a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), demonstrated the existence of two forms of CD23 by releasing its soluble form (sCD23) from membrane CD23 (mCD23). Upregulation of mCD23 in tonsil B cells following treatment with IL-4 and anti-CD40 led to accumulation of sCD23 in the medium prior to class switching to IgE synthesis. Inhibition of mCD23 cleavage by an inhibitor of ADAM10 or small interfering RNA inhibition of CD23 synthesis suppressed IL-4- and anti-CD40-induced IgE synthesis, but addition of recombinant sCD23 enhanced IgE synthesis. Since this occurred even when mCD23 is protected from cleavage, it seems that IgE synthesis is positively controlled by sCD23, and further, sCD23 binds to cells co-expressing IgE and membrane CD21. These results have been interpreted as membrane-bound IgE and CD21 having a role in the sCD23-mediated positive regulation of IgE synthesis with feedback occurring when the concentration of IgE becomes great enough to allow binding to mCD23, thus preventing further release of its soluble form.

3.2.5 Mast Cells and the MRGPRX2 Receptor: A Comparison of FcεRI- and MRGPRX2-Mediated Degranulation

Human mast cells can be activated by a number of different receptors in addition to the FcεRI and FcεRII receptors. The mast cell receptor, mastocyte-related G-protein-coupled receptor member X2 or MRGPRX2, has been identified as a target for several ligands including the neuropeptides substance P (SP); vasoactive intestinal polypeptide and somatostatin; endothelin 1 (ET1); complement anaphylatoxins C3a and C5a; the cationic agonists compound 48/80 and decapeptides icatibant and cetrorelix; antimicrobial peptides, e.g., cathelicidin; opioids such as morphine; and some benzylisoquinolinium NMBDs. The discovery of MRGPRX2 and its murine ortholog Mrgrp2 begins to define the well-known but previously unexplained responses of mast cells to neuropeptides, some drugs, and other “releasing agents” and identifies the receptor as a trigger for so-called pseudoallergic and neurogenic reactions that occur independently of the FceRI degranulation pathway.

Activation of human mast cells by different activation signals mediated through the FcεRI and MRGPRX2 receptors was studied recently using anti-IgE and the neuropeptide SP. Anti-IgE, which activates mast cells by cross-linking FcεRI-bound IgE, induced strong de novo secretion of PGD2 and PGE2 and several inflammatory cytokines including VEGF and chemokines, whereas SP, via the MRGPRX2 receptor (and producing the same extent of degranulation), triggered small amounts of lipid mediators and little VEGF. Anti-IgE triggered a strong and extended release of intracellular calcium ([Ca^{2+}]i) with a lag of about 5 min between this early increase and the first appearance of extracellular granules. Degranulation then progressed over the next 30 min, but in SP-stimulated mast cells, [Ca^{2+}]i was both rapid and brief, and lag time for initial exteriorization of granules was markedly shortened or sometimes almost absent. With regard to granule size, number, and shape during degranulation, anti-IgE activation elicited compound exocytosis, that is, the formation of heterogeneously shaped granules, formed by granule fusion, about 2.7 times the volume of a larger number of smaller spherical granules formed following SP activation (Fig. 3.11). Differences in intracellular signaling between mast cells activated with anti-IgE and those activated by SP were also apparent. Activation of the AKT/PKC/ IKK-β pathway and SNAP23/STX4 complex formation resulted from FcεRI activation, but AKT, PKC, and IKK-α/β phosphorylation did not follow SP, compound 48/80, C3a, C5a, or ET1 stimulation.

Human mast cells have been divided into two subtypes, those expressing only tryptase, designated MC_T, and those expressing tryptase and chymase, designated MC_TC. MC_T mast cells, found mainly in the lung and gut, essentially cor-
Degranulation rapid and concluded relatively quickly

Limited release of PGE₂
No VEGF released

Degranulation slower and over a longer time period
Greater release of PGE₂
Also cytokines and VEGF

**PHYSIOLOGICAL RESPONSE**

| Rapid, transient and local | Prolonged, robust and inflammatory Response systemic and local |

**DOWNSTREAM SIGNALING**

| ? | AKT PKC activated IKK-β phosphorylated |

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**Fig. 3.11** Activation of human mast cells by different activation signals mediated through the FcεRI and G-protein-coupled MRGPRX2 receptor. Following anti-IgE activation via FcεRI, heterogeneously shaped granules formed by granule fusion are seen. Activation of the MRGPRX2 receptor with, for example, substance P rapidly induces exocytosis involving a larger number of smaller spherical granules. Granules formed and released following FcεRI stimulation are about 2.7 times the volume of granules formed following MRGPRX2 stimulation, and degranulation is far more rapid and shorter lasting in the latter case.
respond to murine mucosal mast cells (MMC), while MC_T/ mast cells occur in the connective tissue and correspond to mouse connective tissue mast cells (CTMC). MC_T/ MMC mast cells lack the MRGPRX2/Mrgprb2 receptor, but it is highly expressed in MC_T/ CTMC mast cells. This knowledge helps to explain some previously not understood clinical observations, for example, why drugs like 48/80, SP, and icatibant activate mast cells when injected into the skin but why MC_T/ MMC mast cells are refractory to these secretagogues and also why such pseudoallergic reactions differ from IgE-mediated reactions provoked by the injection of allergen into the skin of sensitized subjects. In contrast to pseudoallergic skin reactions, IgE-dependent reactions are longer-lasting, and associated symptoms persist due to the release of newly synthesized mediators, in particular pharmacologically potent lipids and cytokines.

Since the identification by McNeil and colleagues in 2015 of the human/mouse mast cell-specific receptor MRGPRX2/Mrgprb2 involved in pseudoallergic drug reactions, some different drug classes including NMBDs (benzylisoquinolines, aminosteroids), opioids, quinolones, and vancomycin have been claimed to constitute an additional mechanism for immediate, but not immune, drug hypersensitivity reactions. Experiments are beginning to show, however, that human and murine mast cells show significant differences in their responses to some drugs. Whereas rocuronium induced degranulation mediated by the mouse ortholog receptor Mrgprb2, MRGPRX2-mediated degranulation was not observed in response to the aminosteroid. Also, in a recent in silico study, several claimed secretagogue agonists for MRGPRX2, including rocuronium, atracurium, ciprofloxacin, moxifloxacin, and levofloxacin, could not be validated as MRGPRX2 agonists even when tested at up to 100 μM (see also Chap. 6, Sect. 6.3.3.9 and Chap. 7, Sect. 7.2.2.3).

Although MRGPRX2 is often regarded as a mast cell-specific receptor, it is expressed by basophils and also by eosinophils but not neutrophils. Among mast cell MRGPRX2 agonists, ciprofloxacin (Chap. 6, Sect. 6.3.3.9) is claimed to upregulate expression of the receptor on both basophils and eosinophils.

The recent demonstration that cultured human mast cells can be passively sensitized by IgE antibodies in the sera of patients allergic to chlorhexidine and subsequently induced to degranulate upon challenge with the drug, raises the possibility that a mast cell activation test for the diagnosis and investigation of drug allergies may be a distinct possibility (see Chap. 4, Sect. 4.6.9).

3.3 Important Mediators of the Type I Immediate Allergic Response

As mentioned above, some mediators of inflammation and anaphylaxis and other physiologically and pharmacologically active substances released from mast cells are preformed and stored in the cytoplasmic granules, while others are newly synthesized. The agents producing the most distinctive and severe symptoms of an allergic reaction are histamine, leukotrienes, and platelet activating factor (PAF), but tryptase (Chap. 4, Sect. 4.5.1), prostaglandin D2 (PGD2), the cytokine TNF, some chemokines, and a diversity of chemotactic, stimulating, and growth factors are also significantly involved.

3.3.1 Histamine

The reader is also referred to Chap. 4, Sect. 4.5.2, for a consideration of the place of histamine in the diagnosis of drug allergies and to Chap. 8, Sect. 8.4.1, for a summary of histamine receptors and their relevance to opioid analgesics.

Histamine (2-(imidazol-4-yl)ethylamine) is one of the most intensely studied molecules in all biological systems. This fact and its apparent myriad physiological and pathological effects are behind a seemingly ever-expanding literature on an extraordinarily broad spread of activities including its role in inflammatory and allergic reactions; many aspects of the immune response; differentiation; cell proliferation; hematopoiesis; neurotransmission; regulation of circulatory functions, vasodilation, and blood pressure; wound healing; gastrointestinal function; and perhaps others yet to be elucidated. In peripheral tissues, more than 90%
of body stores of histamine are found in mast cells and basophils although there are two other main sources in humans – enterochromaffin-like cells of the gut and histaminergic nerves in the brain. In mast cells and basophils, histamine is stored in granules in association with different anionic proteoglycans – heparin in mast cells and chondroitin-4-sulfate in basophils. Upon degranulation elicited by specific IgE antibodies, cytokines, or histamine releasers like compound 48/80, calcium ionophore, N-formyl-met-leu-phe, phorbol 12-myristate 13-acetate, and some drugs such as opioid analgesics and neuromuscular blockers, histamine is released from the granules in large amounts with the associated proteoglycan.

### 3.3.1.1 Histamine Biosynthesis and Metabolism

Histamine is synthesized from \( L \)-histidine exclusively by the inducible enzyme \( L \)-histidine decarboxylase located in the cytosol and widely expressed in the body in various cells including mast cells, basophils, parietal cells, gastric mucosa, neurons, and cells of the central nervous system. The mammalian enzyme requires pyridoxine-5-phosphate as an active site cofactor (Fig. 3.12). Once synthesized, histamine is transported from the cytosol to the secretory granules by vesicular monoamine transporter 2 (VMAT2). \( L \)-Histidine decarboxylase is detectable only in cells producing histamine since it is synthesized only when the mediator is required and degraded as soon as synthesis is terminated. Given histamine’s pronounced physiological actions, its inactivation to metabolites that do not interact with histamine receptors is a requirement. This is achieved by methylation and oxidation. In mammals, histamine is inactivated in two main ways – methylation of the imidazole ring effected by histamine \( N \)-methyltransferase (HMT) and oxidative deamination of the primary amino group catalyzed by diamine oxidase (DAO) to form \( N \)-methylhistamine and imidazole-4-acetaldehyde, respectively (Fig. 3.12). HMT, which is specific for histamine, is present in most tissues and responsible for the inactivation of intracellular histamine. The enzyme catalyzes the transfer of a methyl group from \( S \)-adenosyl-\( L \)-methionine to the secondary amino group of the imidazole ring. DAO is stored in secretory vesicles and expressed mainly in intestinal and kidney epithelial cells. Its release is stimulated by heparin which is liberated together with histamine by activated mast cells. Heparin terminates the action of histamine by inactivating it locally. DAO is also active in the gut where it catabolizes histamine present in some foods, thus preventing it from entering the circulation. The products of histamine inactivation by the two different routes are further metabolized (Fig. 3.12). \( N \)-Methylhistamine is converted to \( N \)-methylimidazole-4-acetaldehyde by mitochondrial monoamine oxidases, and this aldehyde, in turn, is catalyzed by aldehyde dehydrogenases to \( N \)-methylimidazole-4-acetic acid. In the DAO pathway, the first product from the breakdown of histamine, imidazole-4-acetaldehyde, is also catalyzed to the acetic acid derivative by aldehyde dehydrogenase before its subsequent ribosylation for transport and excretion.

### 3.3.1.2 Histamine Receptors H\(_{1}\), H\(_{2}\), H\(_{3}\), and H\(_{4}\)

The physiological and pharmacological effects of histamine are mediated through four different receptors H\(_{1}\), H\(_{2}\), H\(_{3}\), and H\(_{4}\) (see also Chap. 8, Sect. 8.4.1), all members of the seven-transmembrane G-protein-coupled receptor (GPCR) family with amino terminal glycosylation sites and phosphorylation sites for protein kinases A and C. The receptors are widely expressed on different tissues that are responsive to histamine. For the H\(_{1}\) receptor, these tissues include smooth muscle cells of the airways and vasculature, the gastrointestinal tract, cardiovascular system, neutrophils, endothelial cells, T and B cells, hepatocytes, nerve cells, and cells of the genitourinary system suggesting an important role for the autacoid in the modulation of immune, inflammatory, and allergic processes. The H\(_{2}\) receptor is expressed in gastric parietal cells, the central nervous system, vascular smooth muscle, heart, neutrophils, and uterus. H\(_{3}\) receptors appear to be less widely distributed occurring in the central and peripheral nervous systems, while H\(_{4}\) receptors are largely expressed in hemopoietic cells where they modu-
Fig. 3.12  Biosynthesis of histamine from L-histidine by the widely expressed enzyme L-histidine decarboxylase and its metabolism by methylation (via histamine N-methyltransferase) and oxidation (via diamine oxidase)
late eosinophil migration and selective recruitment of mast cells. For signal transduction, the H₁ and H₂ receptors activate G_{q} and G_{s}-coupled proteins, respectively, while both H₃ and H₄ are coupled to, and activate, G_{i/o} proteins.

**Histamine H₁ Receptor**
Pathophysiological effects resulting from stimulation of the H₁ receptor include those responses seen in immediate allergic reactions, viz., redness, itch, swelling, asthma, anaphylaxis, bronchoconstriction, and vascular permeability. The primary activation of the H₁ receptor, a G_{q/11}-coupled protein, proceeds through phospholipase C which catalyzes the formation of inositol-1,4,5-triphosphate (IP₃) and 1,2-diacetylglycerol (DAG) from phosphatidylinositol 4,5-biphosphate (Fig. 3.13). IP₃, released into the cytosol, binds to its receptor in the endoplasmic reticulum causing an increase in cytosolic Ca^{2+} levels. DAG, acting as a second messenger, activates protein kinase C (PKC). This pathway is activated and proceeds in the brain, airways, and intestinal and vascular smooth muscle. H₁ receptor activation in some other tissues can stimulate adenyl cyclase and cAMP formation. The signaling pathways are not yet fully understood, particularly details of the involvement of Ca^{2+}. Some of the resultant responses in vascular endothelial cells after stimulation of the H₁ receptor and elevated intracellular Ca^{2+} levels are permeability changes, synthesis of prostacyclin and platelet activating factor (PAF), and release of von Willebrand factor and nitric oxide (NO).

**Histamine H₂ Receptor**
Whereas H₁ receptors are involved with positive effects, H₂ receptors appear to mainly mediate

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**Fig. 3.13** Summarized comparisons of G-protein coupling and main and alternative signaling pathways for the four histamine receptors H₁, H₂, H₃, and H₄. (See also Table 3.1)
suppressive activities of histamine including gastric acid secretion, heart contraction, cell proliferation, differentiation, and some effects on the immune response. H$_2$ receptors are coupled to the adenylate cyclase as well as the phosphoinositide second messenger systems via separate GTP-dependent mechanisms, but H$_2$-dependent effects, particularly those of the central nervous system, are predominantly mediated through cAMP. It has been shown that receptor binding stimulates activation of c-Fos, c-Jun, PKC, and P70S6 kinase. Alternative signaling pathways have been reported (Fig. 3.13). These include a receptor-mediated increase in intracellular Ca$^{2+}$ and/or IP$_3$ levels in HL-60 human promyelocytic leukemia cells and an increase in cAMP and inhibition of release of arachidonic acid in Chinese hamster ovary (CHO) cells transfected with rat cDNA and induced by calcium ionophore.

**Histamine H$_3$ Receptor**

The H$_3$ receptor regulates the synthesis and release of histamine and also has a regulatory role in the release of neurotransmitters such as serotonin, dopamine, and norepinephrine. The receptor is expressed in those regions of the central nervous system associated with cognition, in particular, the hippocampus, basal ganglia, and cortical areas and in the peripheral nervous system, namely, the cardiovascular system, gastrointestinal tract, and airways. The H$_3$ receptor signals through G$_{i/o}$ proteins and alternative signaling pathways appear to be activated by these proteins. Stimulation of the receptor results in adenylyl cyclase inhibition and lower levels of cAMP and PKA. Alternative signaling pathways may be activated including activation of phospholipase A$_2$ (PLA$_2$), stimulation of mitogen-activated protein kinase (MAPK), the inhibition of Na$^+$/H$^+$ exchange, and K$^+$-induced Ca$^{2+}$ mobilization. A study of H$_3$ receptor-mediated attenuation of norepinephrine exocytosis in cardiac sympathetic nerves identified a novel pathway in which stimulation of the receptors on nerve endings produces intraneuronal activation of the MAPK cascade. PLA$_2$, phosphorylated by MAPK, translocates to the cell membrane where it acts on membrane phospholipids producing arachidonic acid, the substrate for cyclooxygenase and the production of prostaglandin E$_2$ (PGE$_2$). PGE$_2$ activates prostaglandin E receptor 3 (EP$_3$R) on the cell membrane where the G$\beta_\gamma$ subunit of EP$_3$R inhibits Ca$^{2+}$ entry resulting in attenuation of norepinephrine exocytosis. It is apparent that with the H$_3$ receptor, different signaling can be employed in different cell systems. A further illustration of this is the demonstration of H$_3$ receptor-mediated activation in the inhibition of the growth of cholangiocarcinoma in vitro and in vivo. Activation of H$_3$ receptors by a high affinity H$_3$ agonist decreased cholangiocarcinoma growth by increasing levels of IP$_3$, translocation of PKC$\alpha$, and IP$_3$/Ca$^{2+}$-dependent dephosphorylation of the extracellular signal-regulated kinases ERK 1/2.

A new signaling pathway of the H$_3$ receptor involving receptor modulation of the activity of the serine-/threonine-specific protein kinase Akt (protein kinase B, PKB)/GSK-3$\beta$ (glycogen synthase kinase 3$\beta$) axis was recently demonstrated in SK-N-MC cells from a neuroepithelioma cell line. Receptor stimulation with an H$_3$ agonist induced the phosphorylation of Ser473 and Thr308 on Akt, a kinase important for neuronal development and function. Studies suggested that the Akt activation occurs via a G$_{i/o}$-mediated activation of PI3K (see Sect. 3.4.2). H$_3$ receptor activation also resulted in phosphorylation of Ser 9 on GSK-3$\beta$, a ser/thr kinase which acts downstream of Akt. This kinase is important in brain function, and this newly identified signaling pathway adds important knowledge to our understanding of the role of H$_3$ receptor-controlled histamine in brain function. The three above-outlined alternative pathways are summarized in Fig. 3.13.

**Histamine H$_4$ Receptor**

Following the realization that not all of the biological effects of histamine could be attributed to histamine receptors H$_1$, H$_2$, and H$_3$, a fourth receptor was postulated, and histamine receptor H$_4$ was subsequently cloned in 2000–2001. Receptor H$_4$ shows a 35% amino acid sequence homology with the H$_3$ receptor, and the two are similar in gene structure. The receptor essentially
confined to hemopoietic cells, exerts a chemotactic effect on several cell types associated with immune and inflammatory responses such as allergy, asthma, rheumatoid arthritis, and inflammatory bowel disease, and this has led to interest in the development of new agents targeting these diseases. H₄ receptors are functionally expressed on mast cells, eosinophils, monocytes, dendritic cells, and CD8+ T cells. Although the presence of large amounts of histamine in mast cells and the cell’s histamine-releasing properties are well-known, expression of histamine receptors on mast cells had not been convincingly demonstrated, and there has been little information of the effect of histamine on the cell. It is now known that mast cells express the H₄ but not the H₁ receptor, but exposure to histamine, or histamine in combination with antigen-IgE antibody complexes, does not lead to degranulation of mast cells. The H₄ receptor has, however, been clearly implicated in inflammation and pruritus in animal models. In a rat model of carrageenan-induced acute inflammation, antagonists of the receptor inhibited edema formation and reversed the thermal hyperalgesia. In a histamine-induced itch model in mice, H₄ antagonists inhibited but did not abolish scratching, and itch was reduced in H₄-deficient mice. Centrally acting H₁ receptor antagonists produced a partial reduction and combined treatment with both antagonists completely eliminated itch. Further evidence for the involvement of both H₄ and H₁ receptors in histamine-induced itch was the production of itch following administration of agonists of both receptors. There are many mediators of itch and mechanisms are complex (see Sect. 3.13). With the belief in mind that the mechanisms underlying itch in chronic conditions such as atopic dermatitis are more likely those associated with mast cell degranulation, a mouse model of itch was set up by injecting antigen-specific IgE intradermally and challenging with antigen 24 h later. H₄ receptor antagonists significantly reduced itch, and this was also the result seen in mice deficient in the H₄ receptor. Interestingly, expression of the H₄ receptor on mast cells or any other cell was not required for the pruritic activity leading to the speculation that H₄ receptor-mediated pruritus may result from actions on peripheral neurons. While the relevance to pruritus in humans of the results with animal models is uncertain, there is optimism that antihistamines specifically targeting the H₄ receptor may lead to more effective treatment of pruritic conditions in humans.

The H₄ receptor is mainly coupled to G_ia/o proteins, and, in common with the H₃ receptor, this leads to inhibition of adenyl cyclase and decreased production of cAMP and downstream effects on cAMP response element-binding (CREB) gene transcription. As with the other histamine receptors, other signaling pathways have been reported (Fig. 3.13). From a study of the signaling pathways of the endogenous mouse H₄ receptor of bone marrow-derived mast cells, histamine activation of the receptor was shown to induce chemotaxis without affecting degranulation of the mast cells. The following interpretations and sequence of events were suggested. Binding of histamine to the receptor on mast cells and eosinophils activates the pertussis toxinsensitive G_ia/o proteins triggering PLC possibly via the G protein βγ subunits dissociated from the G_ia/o proteins. PLC hydrolyzes phosphatidylinositol 4,5-biphosphate to IP₃ and DAG. IP₃ diffuses into the cytosol and binds to its receptor on the endoplasmic reticulum where it activates a Ca²⁺ channel causing the release of intracellular Ca²⁺. The increased Ca²⁺ triggers mast cell chemotaxis toward histamine by pathways yet to be worked out. It has been suggested that this mechanism might be responsible for mast cell accumulation in allergic tissues.

There is accumulating evidence that when the same receptor can activate more than one pathway, some agonists can activate one pathway in preference to another. The need to consider more than one downstream signaling pathway in histamine-GPCR studies was again reinforced by an investigation of signaling at the H₄ receptor using the selective antagonist for G protein-dependent signaling JNJ7777120 (1-[(chloro-1H-indol-2-yl)carbonyl]-4-methyl-piperazine). Downstream signaling measurements of G protein activation and β-arrestin recruitment demonstrated that the antagonist is what has been described as a biased agonist, acting as an agonist
in a non-G protein-dependent manner to recruit β-arrestin to the receptor. β-Arrestin is part of the mechanism for regulating the activity of GPCRs. In stabilizing an alternative active conformation of the H₄ receptor that initiates β-arrestin recruitment but not G protein activation, that is, agonist-biased signaling, JNJ7777120 may be exhibiting the capacity to exist in multiple active conformations. This may result in an agonist stabilizing a slightly different state that preferentially couples to one pathway and not another.

Summarized comparisons of functions, indications for antagonists, G protein-coupling, and signaling pathways for the four histamine receptors are shown in Table 3.1 and Fig. 3.13.

### 3.3.2 Cysteinyl Leukotrienes

For a discussion of cysteinyl leukotrienes in relation to diagnostic investigations of suspected drug allergies, see Chap. 4, Sect. 4.5.3.

Originally isolated after stimulation of lung tissue by histamine and snake venom and named over 70 years ago as “slow reacting substance of anaphylaxis” (SRS-A), leukotrienes are a family of bioactive peptide-conjugated eicosanoid lipids produced by mast cells, basophils, eosinophils, and macrophages. The name “cysteinyl leukotrienes” is derived from the facts that the compounds are synthesized by leukocytes, they contain three conjugated double bonds or alkenes and four members of the group, and LTC₄, LTD₄, LTE₄, and LTF₄ contain the amino acid cysteine. Although the leukotrienes were originally identified by their contractile effect on smooth muscle, they are now recognized as potent inflammatory mediators with a range of other biologic effects. In particular, LTC₄ and LTD₄ are powerful mediators of asthma, airway hypersensitivity, and allergies, inducing bronchoconstriction, increasing vascular permeability and promoting mucous secretion. Upon inhalation, both mediators are up to 1000 times as potent as histamine, whereas LTE₄ is only 39 times as potent as histamine in reducing maximum expiratory flow at 30% of vital capacity. LTE₄, the most stable of the three cysteinyl leukotrienes, is present in greatest amount in vivo where it induces bronchial eosinophilia and airway hyperresponsiveness. Unlike LTC₄ and LTD₄, LTE₄ persists longer in serum, urine, and bronchoalveolar lavage fluid of asthmatics. Urinary excretion of LTE₄ is therefore sometimes used as an indicator of asthma. The bronchoconstriction provoked by LTE₄ is strong in patients with aspirin-sensitive asthma but much weaker in other asthmatics, whereas LTD₄ is much more pronounced in asthmatic patients not sensitive to aspirin (see Chap. 9). Another difference between the two mediators in their effects on asthmatics is the recruitment into sputum of basophils, mast cells, and eosinophils by LTE₄ but not by LTD₄. LTD₄ aids the adhesion and migration of some cancer cells and increases proliferation of mast cells. All three cysteinyl leukotrienes produce an equiactive wheal and flare reaction characteristic of an allergic response when injected intradermally at a concentration of 1 nmol per site.

| Table 3.1 | Summarized comparison of function, G-protein coupling, and signaling pathways of histamine H₁, H₂, H₃, and H₄ receptors⁴ |
|---|---|---|---|---|
| Receptor | H₁ | H₂ | H₃ | H₄ |
| **Best characterized function** | Acute allergic reaction | Gastric acid secretion | Modulation of neurotransmitters | Immunomodulation |
| **Indications for antagonists** | Allergy, pruritus⁵ | Gastroesophageal reflux disease, peptic ulcer⁵ | Sleep and cognition disorders⁶ | Pruritus, asthma⁷ |
| **G-protein coupling** | Gαᵢ/₁₁ | Gαᵢ | Gαᵢ/o | Gαᵢ/o |
| **Main signaling pathway** | Ca²⁺ † | cAMP † | Inhibition of cAMP | Ca²⁺ † |

⁴See also Fig. 3.13  
⁵Approved indications  
⁶Potential indications
3.3.2.1 Cysteinyl Leukotrienes 

Biosynthesis

As part of the response to leukocyte cell activation, cysteinyl leukotrienes are generated de novo from arachidonic acid liberated from cell membrane phospholipid by cytosolic phospholipase A2 (Fig. 3.14). In concert with 5-lipoxygenase-activating protein (FLAP), the enzyme 5-lipoxygenase (5-LO) coverts arachidonic acid to 5-hydroxyperoxyeicosatetraenoic acid (5-HPETE) which spontaneously reduces to 5-hydroxyeicosatetraenoic acid (5-HETE). 5-LO converts 5-HPETE to leukotriene A₄, an unstable peroxide. Note that the enzyme involved in this step is sometimes referred to as LTA₄ synthase. LTA₄ synthase activity co-purifies with 5-LO, and the same cytosolic and membrane-bound active proteins are required for reactions catalyzed by 5-LO and the so-called LTA₄ synthase in crude human leukocyte homogenates leading to the conclusion that a single enzyme is responsible for the production of 5-HPETE from arachidonic acid and for its subsequent conversion to LTA₄. In neutrophils and monocytes which have the enzyme LTA₄ hydrolase, LTA₄ is converted to the dihydroxyacid leukotriene LTB₄, a chemoattractant for neutrophils, whereas in mast cells, basophils, eosinophils, and macrophages, all of which express LTC₄ synthase, LTA₄ is conjugated to reduced tripeptide glutathione to form the cysteinyl leukotriene LTC₄. After transportation to the cell surface in an energy-dependent step with the assistance of multidrug resistance-associated protein 1 (MRP-1), LTC₄ is converted extracellularly to LTD₄ by a γ-glutamyl transpeptidase (γ-GT) or γ-glutamyl leukotrienease (γ-GL). In the final step in the pathway, a dipeptidase removes glycine from LTD₄ producing LTE₄ which is excreted unchanged in the urine. LTF₄ which has an S-glutamylcysteinyl group has been

![Fig. 3.14](image-url)  
Biosynthesis of cysteinyl leukotrienes from arachidonic acid showing the pathways to the formation of LTA₄, LTB₄, LTC₄, LTD₄, and LTE₄
prepared in vitro from LTE₄ with glutathione and γ-glutamyltranspeptidase, but, as yet, it has not been found in vivo. In comparison to the other cysteinyl leukotrienes, LTE₄ contracts vascular smooth muscle poorly – the rank order of potency being LTD₄ > LTC₄ > LTE₄ > LTE₄. Although leukotriene synthesis generally proceeds via the 5-LO pathway, a second family of leukotrienes (eoxins, given the prefix EX) can be generated from the action of 15-LO (and 12-LO) first on arachidonic acid and then, for the 15-lipoxygenase compounds, 15-HPETE to form the 15-epoxytriene 15-LTA₄ (EXA₄) followed by the proinflammatory cysteinyl 15-leukotrienes 15-LTC₄ (EXC₄), 15-LTD₄ (EXD₄), and 15-LTE₄ (EXE₄) in eosinophils, mast cells, and nasal polyps of allergic subject (see also Chap. 9, Sects 9.4.1, 9.4.3 and Figs. 9.3 and 9.4). IL-4-primed human mast cells incubated with arachidonic acid synthesize and release EXC₄ and possess the capacity to produce EXD₄ cells, while nasal polyps spontaneously release EXC₄. Eoxins modulate and enhance vascular permeability, being 100 times more potent in this respect than histamine and almost as potent as LTC₄ and LTD₄. Two types of the 15-LO enzyme are known, 15-LO-1 (which also has about 10% 12-lipoxygenating activity) and 15-LO-2 both of which produce 15(S)-HETE from arachidonic acid but 15-LO-1 oxygenates arachidonic acid at carbons 15 and 12 while 15-LO-2 adds oxygen only at carbon 15. Human eosinophils and airway epithelial cells contain high amounts of 15-LO-1 as do some subsets of human mast cells, macrophages, and dendritic cells. Expression of 15-LO-2 appears to be restricted to the lung, skin, prostate, and cornea.

3.3.2.2 Cysteinyl Leukotriene Receptors

Two human cysteinyl leukotriene receptors CysLT₁R and CysLT₂R, cloned at the turn of the century, do not bind the three cysteinyl leukotriene ligands equally. The rank order of binding for CysLT₁R is LTD₄ > LTC₄ = LTE₄ and for CysLT₂R, LTC₄ = LTD₄ > LTE₄. The receptors are expressed on a wide range of organ tissues and cell types – CysLT₁R on the spleen, lung, small intestine, placenta, bronchial smooth muscle, mast cells, neutrophils, eosinophils, macrophages, monocytes, and hemopoietic progenitor cells and CysLT₂R on the lung, heart, lymph node, spleen, brain, bronchial and coronary smooth muscle, adrenal medulla, mast cells, eosinophils, macrophages, and monocytes. The receptors were initially studied with prototypes of the later-to-be-developed “lukast” antagonists. CysLT₁R, which bound LTD₄ much more strongly than LTC₄, was competitively blocked by the antagonists, while CysLT₂R bound the two cysteinyl leukotrienes with equal affinity and bound LTD₄ at one tenth the affinity shown by CysLT₁R. LTE₄, however, did not display any appreciable binding to either receptor. Despite this, some early recognized pharmacologic properties of LTE₄, viz., its superior potency to its related compounds in contracting guinea pig tracheal smooth muscle and enhancement of this effect produced by histamine, its known peripheral and central airway effects in guinea pigs, and its capacity to increase permeability in guinea pig and human skin, all suggested a distinct pathobiologic role and the existence of a distinct receptor for LTE₄. Studies by K. Frank Austen’s group of cysteinyl leukotriene-dependent swelling of ear tissue in mice lacking both CysLT receptors proved the existence of a distinct LTE₄-reactive cutaneous receptor. Ear swelling, a measure of LTE₄-mediated vascular leakage, was inhibited by pretreatment with pertussis toxin or a Rho kinase inhibitor indicating the involvement of a human GPCR to Ga proteins and Rho kinase. Until this cutaneous receptor is cloned, it has been designated CysLT ER. Further studies of single receptor-deficient strains of mice compared to wild-type mice showed that the permeability response to LTC₄ or LTD₄ was reduced by half in Cslt₁r⁻/⁻ mice (mice lacking the two receptors) but was normal in magnitude and delayed in Cslt₂r⁻/⁻ mice. These results suggested that CysLT₁R is the major signaling receptor for LTC₄ and LTD₄, while CysLT₂R negatively regulates CysLT₁R. Vascular leakage was not reduced by LTE₄ in Cslt₁r⁻/⁻ mice but again sustained and delayed in the Cslt₂r⁻/⁻ strain indicating that CysLT₂R is the dominant receptor for
LTE₄ and that CysLT₂R once again acts as a negative regulator. Studies on expression of cysteinyl leukotriene receptors by human mast cells unexpectedly revealed that LTE₄ helps to induce greater numbers of mast cells from cord blood progenitor cells cultured together with IL-6 and IL-10 than both LTC₄ and LTD₄ and it is also more potent for the production of the inflammatory chemokine macrophage inflammatory protein-1β (MIP-1β) and for the expression of COX-2 and prostaglandin D₂. Sequence homologies of the classical type I and II cysteinyl leukotriene receptors and the P2Y receptor family together with computer modeling studies indicated that LTE₄ might be a surrogate ligand for a previously unrecognized receptor on mast cells. Human mast cells express the P2Y₁₂ receptor, a Gαi-linked receptor for adenosine diphosphate. In subsequent investigations by Austen’s group using ovalbumin-sensitized and Cyslt₁r/Clt₂r −/− mice (mice lacking the two receptors), the expression of IL-13 and use of the P2Y₁₂ receptor-selective antagonist clopidogrel suggested that LTE₄ acted as an agonist for platelet activation in the pulmonary vasculature. It seems, therefore, that P2Y₁₂ is the receptor for LTE₄-mediated amplification of allergic pulmonary infiltration and proliferation of mast cells and this receptor is separate and distinct from the CysLT₂R in the skin.

A number of observations supported the possibility of an agonist function for LTE₄ mediated by a receptor separate from the two known receptors. These observations were the following: (1) pretreatment of guinea pig trachea and human bronchial smooth muscle with LTE₄, but not LTC₄ or LTD₄, enhanced the response of these tissues to histamine; (2) all three cysteinyl leukotrienes showed comparable activity in eliciting vascular leakage in the skin; and (3) inhalation of LTE₄, but not LTD₄, by asthmatics led to an accumulation of basophils and eosinophils in the bronchial mucosa.

With the aim of investigating the existence of a potential third cysteinyl leukotriene receptor, Austen and colleagues, using Cyslt₁r/Clt₂r −/− mice, observed a more pronounced edema response to LTE₄ than to LTC₄ and LTD₄ following intradermal injection of each agent, and the response to LTE₄ was 64 times more potent in the Cyslt₁r/Clt₂r −/− mice than in wild-type mice. By screening cDNAs from the P2Y receptor family, the oxoglutarate receptor (Oxgr1) GPR99 was implicated as a potential third leukotriene receptor. In particular, vascular permeability resulted in Gpt99 −/− mice following intradermal injection of LTC₄ and LTD₄ but not LTE₄, and vascular leak was not seen in Gpt99/Cyslt₁r/Clt₂r −/− mice to any of the three ligands. It seems clear that CysLT₃R/GPR99 has high affinity for LTC₄ and is predominately an epithelial cell receptor. Other studies have shown that bronchoconstriction induced in asthmatics by LTC₄ is associated with increases in urine of metabolites of the COX pathway, PGD₂, as well as PGE₂ and PGF₂α and thromboxane A₂. These results are of added interest since they show that, via its receptor, LTC₄ induces the formation of COX products, some of which are derived from mast cells.

### 3.3.3 Platelet-Activating Factor

Platelet-activating factor (PAF), a mediator of anaphylaxis released by mast cells, is one of the most powerful autacoids yet discovered. The PAF story began in the early 1970s when Benveniste, Henson, and Cochrane demonstrated the release of a substance with both powerful anaphylactic and platelet aggregating properties from allergically sensitized rabbit leukocytes. Although first investigated in relation to anaphylaxis and other allergic manifestations, later studies revealed a wide diversity of other biological actions and involvement in diseases such as asthma, some delayed hypersensitivity reactions, septic shock, adult respiratory distress syndrome, rheumatoid arthritis, necrotic bowel disease, and a wide range of other inflammatory conditions. This diversity of biological actions and pathogenic involvements is due to the mediator’s activation of other cells besides platelets, in particular, eosinophils, neutrophils, fibrocytes, neurocytes, and endothelial, vascular, cardiac, smooth muscle, pancreatic, and secretory cells.
3.3.3.1 Chemistry and Structure-Activity Relationships of PAF

PAF, 1-\(O\)-alkyl-2-acetyl-\(sn\)-glycero-3-phosphocholine, a phospholipid of relatively simple but unique structure, belongs to a relatively minor class of lipids, the ether-linked phospholipids. The distinguishing feature of its unique structure is an acetyl group at position 2 of the glycerol backbone (Fig. 3.15). Removal of the acetyl group produces lyso-PAF which is devoid of biological activity. When produced and liberated naturally in the cellular environment, PAF is made up of a mixture of homologues differing in the number of carbons and the degree of unsaturation of the alkyl chain at position 1 of the glycerol backbone. The main homologues usually present are the \(C_{16:0}\), \(C_{18:0}\), and \(C_{18:1}\) structures. The structures for maximum activity are a 16 carbon chain, the 1-\(O\)-alkyl ether linkage, the acetyl group at position 2, the \(sn\) configuration, and the phosphate group at carbon 3 (Fig. 3.15). Activity decreases progressively as the C chain backbone is shortened or lengthened; replacement of the ether linkage leads to no or little bio-

![Fig. 3.15](a) Two-dimensional structure of PAF highlighting the important structural features necessary for maximum biological activity and recognition by anti-PAF antibody combing sites. (b) Three-dimensional space-filling CPK model of PAF with the acetyl group circled. Removal of this group alone produces a molecule devoid of biological activity. (c) Outline of PAF model indicating regions of excellent, good, and poor recognition by anti-PAF antibodies. The antibody recognition pattern is very similar to that of the PAF receptor. (See Smal MA, Baldo BA and Harle DG. J Mol Recogn. 1990;3:169–73.)
logical activity; the unnatural enantiomer with the \((S)\)-configuration is inactive; for any biological activity, the only substituents tolerated at carbon 2 are propionyl and \(N\)-methyl carbamoyl groups (the 2-ethoxy analog has only 10\% of the activity of PAF); and fairly major modifications of the substituents on the nitrogen diminish activity. These specific structural requirements suggest that PAF exerts its biological effects by binding to specific receptors and this is in fact so (Sect. 3.3.3.4).

### 3.3.3.2 Biosynthesis and Cellular Sources of PAF

Because PAF is such a potent mediator of a range of biological effects, its concentration in body fluids and tissues needs to be restricted to avoid adverse or even lethal consequences. This is achieved intracellularly and extracellularly by a specific acetylhydrolase and by regulation of the conversion of precursor molecules. The activity of PAF acetylhydrolase for its substrate is extremely high ensuring that the half-life of the mediator in blood is of the order of only a few minutes. PAF is synthesized by two metabolic pathways – the de novo and remodeling pathways. In the de novo pathway (Fig. 3.16a), the specific enzyme alkylacylglycerol cholinephosphotransferase, widely distributed in tissues on the cytoplasmic surface of the endoplasmic reticulum, catalyzes the reaction between 1-\(O\)-alkyl-2-acetyl-\(sn\)-glycerols and cytidinediphosphocholine (CDP-choline) in the presence of \(Mg^{2+}\) generating PAF and cytidine monophosphate. This synthetic pathway appears to maintain PAF levels for normal physiological processes. The remodeling pathway (Fig. 3.16b) both activates and deactivates PAF via the calcium-dependent enzymes phospholipase A2 and acetyltransferase, the latter being the rate-limiting enzyme. These enzymes are found particularly in cells of the immune system.

![Fig. 3.16](image-url) The two biosynthetic pathways for the synthesis of PAF. (a) The de novo pathway; (b) the remodeling pathway.
system such as basophils, eosinophils, platelets, polymorphonuclear cells, macrophages, and endothelial cells and can be stimulated by a variety of agents including immune complexes, thrombin, and histamine.

### 3.3.3.3 Biological Actions of PAF and Its Role in Health and Disease

PAF is a hydrophobic molecule, and for crossing cell membranes and transportation to its various sites of action, serum albumin serves a carrier function. When injected into mammals, PAF produces both the signs and symptoms of anaphylaxis with hypotension, increased vascular permeability and hemoconcentration, thrombocytopenia, neutropenia, and eventually death. Infusion of PAF into the heart decreases myocardial contractility and coronary flow, effects resembling cardiac anaphylaxis. Intradermal injection produces a biphasic inflammatory response similar to the response of allergic subjects to allergen. PAF has a profound effect on the lung producing bronchoconstriction, edema, and hyperresponsiveness. PAF is also one of the most powerful ulcerogenic agents known, provoking hemorrhage and vascular congestion in both the stomach and small intestine.

PAF has been implicated in many disease states, but since it is often only one of a range of other mediators present, any pre-eminent role is understandably often difficult to establish. For example, it is frequently present along with histamine, numerous metabolites of the cyclooxygenase and lipoxygenase pathways, and a range of chemokines and cytokines including TNF. As well as its undoubted role in anaphylaxis and some other allergic reactions, PAF is an important mediator in the asthmatic response. Administration of PAF into the lungs produces severe bronchoconstriction, mucous secretion, inflammation, and long-lasting airway hyperreactivity. The latter two effects may be contributed to by PAF-induced recruitment and activation of inflammatory cells such as macrophages and eosinophils. Recent findings, particularly in studies in the mouse, have identified a second pathway of anaphylaxis involving the IgG receptor FcγRIII and the release of PAF as the major mediator (Sects. 3.4.2 and 3.4.4). Although long suspected, a central role for PAF in anaphylaxis is explained and proven by this alternative pathway, but an equivalent involvement in human anaphylaxis has yet to be confirmed. Some data in support of such a second pathway is the finding that PAF, unlike histamine and tryptase, showed a good correlation with severity scores when these mediators were measured in blood from 41 patients during acute allergic reactions. Additional support for the direct correlation of serum PAF levels with the severity of anaphylaxis was the demonstrations that serum PAF acetylhydrolase activity was inversely correlated with severity and the enzyme was significantly lower in patients with peanut-induced fatal anaphylaxis than in controls. The authors surmised that inactivation of PAF by PAF acetylhydrolase may decrease its concentration which, in turn, lessens the likelihood and the severity of anaphylaxis.

In summary, the lines of evidence pointing to an important role for PAF in anaphylaxis include the following: PAF is produced in humans and experimental animals during episodes of anaphylaxis; PAF produces the signs, symptoms, and physiological changes seen in anaphylaxis; PAF levels correlate with the severity of anaphylaxis; inhibitors of PAF prevent anaphylaxis; pretreatment with PAF acetylhydrolase can lessen the severity of, or perhaps even prevent, anaphylaxis; mice with a defective PAF receptor do not experience anaphylaxis; and epinephrine can disrupt PAF signaling and its early use clinically can prevent fatal anaphylaxis. Note that by themselves, and even collectively, these points do not establish unequivocally a central role or prime importance for PAF in human anaphylaxis.

In a model of peanut allergy, although both histamine and PAF are involved in the response, PAF is more important in shock pathogenesis. Along with anaphylaxis and asthma, septic shock is a disease in which PAF is suspected of having a leading role. PAF induces systemic responses similar to those provoked by bacterial endotoxin and is found in the spleen and peritoneum of rats with endotoxic shock. Some PAF antagonists protect animals against septic shock caused by
infection with Gram-negative organisms or purified endotoxin. Because of its potent effect on platelets, PAF is thought to be involved in some thrombotic diseases including stroke. Other suspected roles are in acute graft rejection and immune complex deposition, for example, systemic lupus erythematosus, psoriasis, and a variety of other allergic conditions.

3.3.3.4 The PAF Receptor
The PAF receptor is a MW 48 kD, G-protein-coupled single 342 amino acid protein that shows structural characteristics of the rhodopsin gene family. The human, guinea pig, and rat receptors have been cloned and characterized as a seven-transmembrane receptor coupled to guanine nucleotide-binding proteins (G proteins) initiating and modulating signaling including inhibition of adenyl cyclase and activation of phospholipases A2, C, and D, MAP kinase, tyrosine kinases, and phosphatidylserine-3-kinase. The receptor shows wide tissue distribution being expressed in the lung, kidney, liver, spleen, small intestine, and brain. In leukocytes, it is expressed on platelets, neutrophils, monocytes, and B cells but not on resting T cells and natural killer cell lines. Human monocytes treated with IFN-γ show a two- to sixfold increase in PAF receptor expression compared to untreated cells. In addition to the involvement of different G proteins in signal transduction, receptor binding studies suggest that subtypes of the PAF receptor with different affinities may exist.

3.3.3.5 Measurement of PAF in the Laboratory
Accurate measurement of the very small amounts of PAF in fluids and extracts is a prerequisite for studying the role of the mediator in health and disease. The most widely used method relies on the interaction of PAF with platelets, but the procedures are not strictly quantitative, lack specificity, and are difficult to standardize and reproduce, fresh platelet suspensions are required, and throughput capacity is poor. Mass spectrometric techniques are sensitive and specific, but the specialized nature of the equipment, absence of easy access for many laboratories, and difficulty of assessing large numbers of samples side by side make this method problematic for routine use by many researchers. Other methods such as measurement of 3H-serotonin after PAF-induced platelet degranulation and radioreceptor assays are specialized procedures in some laboratories but can be difficult to standardize, require cell labeling or membrane preparations, and may show high non-specific binding. Specific immunonoassays can provide high throughput procedures for quantitating PAF levels in research and diagnostic test samples. Requirements for such an assay are precise specificity for PAF with no unwanted cross-reactivity and not affected by inhibitors of PAF, good sensitivity, reliability, and ease of use. Perhaps the best sensitive and specific assay with a well-defined and mapped antibody combining site satisfying these requirements is the PAF radioimmunoassay developed in the authors’ laboratory in 1989. The method is sensitive over the range 10–1000 pg (0.02–2 pmoles), has a high capacity, and is highly specific for PAF. Most importantly, lyso-PAF which lacks the acetyl group and occurs in fluids and tissues in much larger quantities than PAF, is without inhibitory activity at 100 ng/ml and very weakly inhibitory in the μg/ml range. Likewise, all the commonly occurring lipids were non-inhibitory up to 20 μg/ml.

3.4 Anaphylaxis

For the clinical features of anaphylaxis, the reader is referred to Chap. 2, Sect. 2.2.1.

Anaphylaxis is a sudden, systemic reaction involving several different organs of the body that may be severe enough to cause death. It is usually provoked by exposure to allergens with drugs, foods, and venoms being the most common causes. Progress continues in identifying key intermediates and elucidating mechanisms of regulatory systems and signaling pathways during mast cell activation and degranulation (Sect. 3.2.2). Impressive advances in our understanding of the pathways, the mediators involved, and their contribution to the pathobiology of anaphylaxis are continuing.
3.4.1 Humoral and Cellular Effectors of Anaphylaxis

Mechanisms underlying an anaphylactic reaction can differ between species and by the concentrations of IgG antibodies involved. In humans, the dominant components in an anaphylactic reaction are small amounts of IgE antibodies and allergen, mast cells and basophils with the FceRI receptor, and often low concentrations of IgG. In mice, however, relatively large concentrations of IgG antibodies and antigen, plus neutrophils, macrophages, and basophils, as well as mast cells, with FcγR, are known to induce anaphylaxis (Sect. 3.4.4; Fig. 3.17). Histamine is an important mediator of anaphylaxis in both species. CysLTs, PAF, prostaglandins, and heparin have been found to be involved in murine anaphylaxis, but, despite some evidence, their role in human anaphylaxis is not yet completely clarified. Evidence for the involvement of proteases and serotonin in both species is far from established.

The main effector proteins, cells, and pharmacologically active small molecule mediators of anaphylaxis are summarized in Table 3.2. In mice, in addition to the classical pathway mediated by FceRI, mast cells, histamine, and PAF, anaphylaxis can occur via an IgG-FcγRIII-macrophage-PAF pathway. With the qualification of some reactions to dextrans (Chap. 7, Sect. 7.4.3), there is, as yet, no conclusive evidence for an IgG antibody-mediated mechanism in humans. It has been suggested, however, that up to 30% of cases of drug-induced anaphylaxis are not induced by IgE antibodies, many patients who experience anaphylaxis to a drug do not have
elevated serum tryptase and specific IgE levels, and these markers, unlike PAF, do not necessarily reflect the severity of the anaphylactic event. It has been suggested that IgG antibodies might bind small quantities of antigen but larger quantities, together with complementary IgG, could mediate anaphylaxis (Sect. 3.4.4). Whether this mechanism is operative in humans is still to be decided one way or the other, but it is clear that humans possess the necessary cells, receptors, antibodies, and other biologically active proteins to support such a pathway.

**Table 3.2** Roles of antibodies, complement, effector cells, and mediators in anaphylaxis in humans

| Antibodies | | |
|---|---|---|
| IgE | • Increased IgE levels are present in patients with allergic diseases | |
| &nbsp; &nbsp; Purified IgE can transfer skin reactivity from a sensitized human subject to a naive host | |
| &nbsp; &nbsp; The anti-IgE antibody omalizumab can decrease the risks of anaphylaxis | |
| IgG | • No definitive evidence is present to date | |
| &nbsp; &nbsp; Cases of anaphylaxis were reported after treatment with therapeutic mAbs without detectable levels of anti-drug IgE<sup>b</sup> | |

| Complement | | |
|---|---|---|
| Anaphylatoxins | • Injection of low doses of C3a, C4a, or C5a in the skin of healthy volunteers induces immediate wheal and flare reactions | |
| &nbsp; &nbsp; Blood levels of C3a, C4a, and C5a correlate with the severity of anaphylaxis in human subjects | |

| Effector cells | | |
|---|---|---|
| Mast cells | • Increased tryptase levels have been detected during acute anaphylaxis in human subjects | |
| &nbsp; &nbsp; There is a high occurrence of anaphylaxis in patients with mastocytosis | |
| Basophils<sup>c</sup> | • There is no definitive evidence to date | |
| &nbsp; &nbsp; Basophil activation tests were used to diagnose or confirm allergen sensitization | |
| Neutrophils<sup>d</sup> | • Myeloperoxidase levels are increased in patients with anaphylaxis compared with healthy donors | |
| Monocytes/macrophages | • Not yet determined | |
| Platelets<sup>e</sup> | • There is no definitive evidence to date | |
| &nbsp; &nbsp; Anaphylaxis in human subjects is associated with platelet activation | |

| Mediators | | |
|---|---|---|
| Histamine | • Aerosol administration of histamine induces bronchoconstriction in healthy volunteers | |
| &nbsp; &nbsp; Intravenous administration of histamine in volunteers can reproduce many of the symptoms of anaphylaxis | |
| &nbsp; &nbsp; H<sub>1</sub> antihistamines are commonly used as adjunctive therapy for acute anaphylaxis and anaphylactoid reactions | |
| CysLTs<sup>f</sup> | • Levels of some CysLTs are increased during anaphylaxis onset | |
| &nbsp; &nbsp; Intradermal injection of LTB<sub>4</sub>, LTC<sub>4</sub>, and LTD<sub>4</sub> induces a wheal and flare reaction in healthy volunteers | |
| &nbsp; &nbsp; Aerosol administration of LTC<sub>4</sub> and LTD<sub>4</sub> in healthy subjects induces bronchoconstriction | |
| PAF | • Injection of PAF in the skin of healthy volunteers induces wheal and flare reactions | |
| &nbsp; &nbsp; Circulating PAF levels increase and circulating PAF acetyl hydrolase activity decreases in proportion to the severity of anaphylaxis | |

From Reber LL, Hernandez JD, Galli SJ. The pathophysiology of anaphylaxis. J Allergy Clin Immunol. 2017;140:335–48. Adapted with permission from Elsevier

<sup>a</sup>Anaphylaxis induces increases in levels of many mediators that could contribute (positively or negatively) to the clinical signs and symptoms. This includes, for example, various cytokines and chemokines, prostaglandins, bradykinin, and serotonin

<sup>b</sup>May occur after larger doses of antigen than required for IgE-mediated anaphylaxis, e.g., after infusion of large quantities of drug or mAb

<sup>c</sup>Express high levels of FcεRI, activating IgG receptor FcγRIIA and inhibitory IgG receptor FcγRIIB. IgE-dependent activation of human basophils is associated with increased levels of basophil surface markers CD203c and CD63

<sup>d</sup>Express several activating FcγRs and can produce histamine and release PAF

<sup>e</sup>Activated platelets can also release platelet factor 4 and serotonin. Express FcεRI, FcεRII, and FcγRIIA

<sup>f</sup>LTE<sub>4</sub>, 2,3-dinor-9α,11β-PGF<sub>2</sub>, and 9α,11β-PGF<sub>2</sub> are increased during the onset of anaphylaxis. Aerosol of LTC<sub>4</sub> and LTD<sub>4</sub> induces bronchoconstriction with 10,000 times the potency of histamine
3.4.2 Intracellular Mechanisms of FcεRI-Mediated Mast Cell Activation in Anaphylaxis

Understanding anaphylaxis involves study of the cellular events leading to the release of mediators of inflammation and hypersensitivity with emphasis on the mechanisms, in particular the signaling processes, of mast cell and basophil activation and degranulation. Upon activation of mast cells and basophils following cross-linking by allergen of receptor-bound IgE and aggregation of the high affinity IgE FcεRI receptors, the cells quickly release preformed mediators from the secretory granules (Sect. 3.2.2). These mediators, including histamine, leukotrienes, PGD2, and TNF, causing vasodilation, increased vascular permeability and heart rate, bronchoconstriction, airway remodeling, pulmonary and coronary vasoconstriction, and a host of other detrimental effects, including cell recruitment with cytokine and chemokine production, are responsible for the pathophysiology of anaphylaxis. Cross-linking initiates the signaling cascade that ultimately results in anaphylaxis. FcεRI receptor aggregation causes Lyn, the tyrosine kinase associated with the β and γ subunits of the receptor, to phosphorylate the tyrosines of the ITAMs of these two subunits. The phosphorylated ITAMS, mainly on the γ subunit, then act as scaffolds for binding the cytoplasmic tyrosine kinase Syk. As outlined above in Sect. 3.2.2, recruitment of the Syk kinase and subsequent phosphorylation activation steps involving Lyn lead to mast cell activation demonstrating the importance of protein tyrosine kinases in the pathways that result in allergic inflammation and anaphylaxis. These pathways involved in mast cell triggering are summarized in a simplified form in Fig. 3.18. Activated Syk is involved in the phosphorylation of the transmembrane adaptor linker for activation of T cells (LAT) as well as the SH2 domain-containing leukocyte-specific protein of MW 76 kD (lymphocyte cytosolic protein 2 LCP2 or SLP-76), the guanine nucleotide exchange factor Vav, and phospholipase C-γ1 (PLC-γ1) and PLC-γ2. After involvement of the enzyme proto-oncogene tyrosine-protein kinase (Fyn), tyrosine phosphorylated GAB2 (GRB2) [growth factor

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Fig. 3.18 Simplified summary of FcεRI-mediated signaling pathways in the mast cell leading to allergic inflammation and anaphylaxis
receptor-bound protein 2-associated-binding protein 2) binds a subunit (p85) of phosphatidylinositol 3-kinase (PI3K). In the membrane, PI3K catalyzes the conversion of phosphatidylinositol-4,5-diphosphate (PIP₂) to phosphatidyl-3,4,5-triphosphate (PIP₃). This attracts a number of proteins containing the pleckstrin homology (PH) domain, a 120 amino acid domain occurring in a variety of proteins involved in intracellular signaling. The attracted proteins include Bruton’s tyrosine kinase (Btk) and the PLCs γ₁ and γ₂ which in tyrosine-phosphorylated form catalyze the hydrolysis of PIP₂ to inositol-1,4,5-triphosphate (IP₃) and 1,2-diacylglycerol (DAG). Both compounds act as second messengers, the former releasing Ca²⁺ resulting in a depletion of Ca²⁺ stores and entry of Ca²⁺ from the extracellular medium and the latter activating protein kinase C (PKC). These events lead to mast cell degranulation. This activity takes place in two regions on the inner side of the plasma membrane. Electron microscopy has revealed a primary region of activity near the FcεRI receptor involving Gab2, the p85 subunit of PI3K, and PLC-γ₂ and a second region near LAT involving PLC-γ₁ and the p85 subunit. Tyrosine phosphorylation and activation of other enzymes and adaptors including Vav, Grb2, the SHC-adaptor protein (Shc) involved in signaling and Son of sevenless (Sos) protein (a guanine nucleotide exchange factor) stimulate the small GTPases Ras, Rho, and Rac. These reactions lead to activation of the extracellular signal-regulated kinase ERK, Jun amino terminal kinase JNK, the p38 mitogen-activated protein (MAP) kinase cascade, and histamine release. Phosphorylation of the transcription factors activating protein-1 (AP-1), nuclear factor of activated T cells (NFAT), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) induces the synthesis of cytokines and the activation of cytoplasmic phospholipase A₂ (PLA-A₂) to release arachidonic acid with the production of lipid mediators (Fig. 3.18).

Further research, much of it in mice, has continued to highlight the key role of tyrosine kinases in FcεRI activation and the subsequent signaling events although other involved tyrosine kinases such as Hck have been identified. The intensity of stimulation of the FcεRI receptor has been shown to be important. Low-intensity stimulation by IgE with low antigen concentration or by anti-IgE positively regulates mast cell degranulation and the production of cytokines by inhibiting Lyn activity. High intensity stimulation with high IgE and high antigen concentrations negatively regulate mast cells by enhancing Lyn activity and increased Syk activation. Genetic variation appears to influence the role of tyrosine kinases. For example, an epilepsy- and anaphylaxis-prone strain of mice was found to be deficient in the expression of Lyn, while a related epilepsy-prone variant proved anaphylaxis-resistant. Bone marrow-derived mast cells (BMMCs) from the anaphylaxis-sensitive mice had reduced Lyn and Syk activities and showed degranulation typical of BMMCs of phenotype Lyn(−/−), whereas the phenotype of the anaphylaxis-resistant mice was similar to wild-type animals.

3.4.3 Sphingosine-1-Phosphate, an Emerging Mediator of Anaphylaxis

Activated Fyn, involved in a second tyrosine kinase pathway, has been shown to be required for cytokine production as well as degranulation and to have a role in generating sphingosine-1-phosphate (S1P) (Fig. 3.19) a blood-borne bioactive lipid mediator that is a major regulator of the
vascular system and B and T cell trafficking. S1P from mast cells and circulating S1P from macrophages, platelets activated by the release of PAF, endothelial cells, and many other non-immune cells are elevated in the lungs of asthmatics where they regulate pulmonary epithelium permeability and are thought to contribute to the pathogenesis of asthma and diseases such as rheumatoid arthritis. The detection of elevated S1P levels in bronchial alveolar lavage fluid of challenged asthmatics and demonstration that it is necessary for sustained mast cell degranulation through the S1P1 receptor was indications that this lysosphingolipid has a previously unrecognized relationship with anaphylaxis. It is produced by sphingosine kinase (Sphk1 and Sphk2)-catalyzed addition of phosphate to sphingosine, broken down by a S1P lyase, and converted back to sphingosine by S1P phosphatase. Recent research has shown that susceptibility to anaphylaxis appears to be due to S1P generated within the mast cell and by free, circulating S1P from non-mast cell sources. The gene SphK2 regulates the influx of Ca2+ into mast cells and the responses to it, making it a determinant of intrinsic mast cell function, whereas SphK1 appears to act extrinsically affecting mast cell responsiveness by regulating levels of circulating S1P. The surprising demonstration of a relationship between circulating levels of S1P and anaphylaxis is made more intriguing by the demonstration that reduced S1P levels due to a deficiency of SphK1 are associated with resistance to anaphylaxis. While it is well-known that only a small number of individuals from a large group with similar circulating levels of allergen-specific IgE antibodies will experience anaphylactic shock when challenged with the allergen, the amount of circulating S1P might help to provide the explanation. Finally, as exciting as these developments in our understanding of the underlying mechanisms of anaphylaxis are, it should be remembered that many of the findings result from research on mice not man, and that is also true for most of the S1P studies where mice with SphK1 and 2 genes, individually or jointly deleted, were used. Given the diversity of mast cells and differences in gene expression of mouse and human mast cells, the roles of SphK1 and SphK2 may prove to be significantly different in the two species.

3.4.4 Other Mechanisms of Anaphylaxis: IgG, PAF, and Nitric Oxide

Mechanisms of anaphylaxis independent of IgE have been suggested, for example, anaphylatoxins produced during complement activation, generation of immune complexes, the involvement of T cell activation and cytotoxicity, release of neuropeptides and a number of different mechanisms acting coincidently without the involvement of allergen-specific IgE. Intriguingly, anaphylaxis can occur in the mouse via the classic pathway involving allergen-induced cross-linking of mast cell FcεRI receptor-bound IgE antibodies with release of histamine (and other mediators) but also by an IgG pathway in which allergen-antibody complexes activate macrophages by cross-linking FcγRIII receptors and with PAF as the main mediator of anaphylaxis. Although there is, as yet, no compelling evidence for an IgG-mediated mechanism in humans, what appears to be anaphylaxis has been described in a few cases where there is an apparent absence of mast cell degranulation, that is, with no increase in serum tryptase. Certainly there are many similarities between the immune systems of mice and men; PAF is produced by macrophages of both species; it has the same effect on vascular permeability, and consequently allergen-IgG complexes may have an important role in anaphylaxis in humans as well as the mouse. However, human anaphylaxis tends to result from low dose exposure, whereas mouse IgG-mediated anaphylactic reactions may occur in response to relatively larger antigen doses and/or adjuvants that elicit IgG as well as IgE antibodies. What may be evidence of anaphylaxis in humans independent of IgE are the responses seen in patients after receiving the chimeric mouse-human anti-TNF monoclonal antibody infliximab (Chap. 13). None of the subjects appeared to have complementary IgE
antibodies, all had IgG to the mouse immunoglobulin determinants, and there was no increase (at only 20 min) in serum tryptase. From insights gained from his extensive studies of mechanisms of anaphylaxis in mouse models, F. D. Finkelman has suggested that large doses of antigen might be used in humans to look for evidence of anaphylaxis accompanied by macrophage activation and PAF secretion.

PAF contributes to hypotension and cardiac dysfunction during shock and stimulates, via its receptor, a number of signaling pathways including those that activate PLA₂ and PI3K. Studies of PAF and anaphylactic shock in mice have shown that PAF-induced shock depends on PI3K signaling and on NO produced by constitutive nitric oxide synthase (eNOS) not the inducible form of the enzyme (iNOS). Mouse models showed that inhibition of NOS, PI3K, or Akt, or deficiency of eNOS, gave complete protection against anaphylaxis. These findings appear to support the belief that eNOS has a detrimental role in vascular function during shock and in regulating inflammation. Further, if eNOS-derived NO is the principal vasodilator in anaphylactic shock, eNOS and/or PI3K or Akt might prove to be important targets for treating anaphylaxis.

In a recent study of the mechanism of anaphylaxis in human low-affinity IgG receptor locus knock-in mice, mice expressing human(h) FcγRIIA/IIB/IId/IIB proved susceptible to passive and active anaphylaxis with hFcγRIIA predominating. Besides FcγRIIA, the activated pathway involved neutrophils and basophils and the mediators PAF and histamine. It was speculated that a similar pathway to anaphylaxis can occur in humans.

3.5 Drug-Induced Urticaria

For the clinical manifestations of urticaria, see Chap. 2, Sect. 2.2.2.

Of the drugs implicated in provoking urticaria and angioedema, the non-steroidal anti-inflammatory drugs (NSAIDs) are perhaps the most important. What is currently understood of their proposed mechanisms of action together with a review of the arachidonic acid cascade is considered in Chap. 9. Formation of the cysteinyl leukotrienes is detailed in Sect. 3.3.2 (above) and is also referred to in Chap. 9.

Urticaria may be classified as acute or chronic. The acute form, more common in children, appears early after exposure, perhaps within minutes, and can last from hours to several weeks, whereas the chronic form, more common in adults, persists for about 6 weeks or more. Chronic urticaria (chronic spontaneous urticaria, chronic idiopathic urticaria), characterized by the rapid appearance of transient, pruritic skin wheals and/or angioedema, is a common, often debilitating disease in which the cause remains unidentified in about 80% of cases. Urticaria is associated with angioedema in about 40–50% of cases. In assessing the severity of the disease, the European Academy of Allergology and Clinical Immunology, Dermatology Section, proposed a score relating the number of wheals to the severity of pruritus: score 0, 0 wheals, no pruritus; score 1 (mild), <20 wheals/24 h, mild pruritus; score 2 (moderate), 21–50 wheals/24 h, moderate pruritus; and score 3 (intense), >50 wheals/24 h or large confluent areas, intense pruritus. Urticaria is often an isolated event, but drug-induced urticaria, regarded as one of the most common cutaneous drug reactions, can be seen in association with anaphylaxis, angioedema, and serum sickness. Urticaria is a heterogeneous disease with many subtypes caused by a range of agents and stimuli. Some infections (e.g., Helicobacter pylori), intolerance to foods, and autoantibodies to the high affinity IgE receptor FceRI have been implicated, but, apart from the NSAIDs, there is a dearth of information on mechanisms underlying drug-induced urticaria. In particular, information on genetic polymorphisms of relevant genes together with supporting functional studies is needed to help elucidate molecular mechanisms and identify genetic markers for urticaria. Some progress made in identifying HLA alleles and promoter polymorphism genetic markers for aspirin-induced urticaria/angioedema is covered in Chap. 9.
3.5.1 Urticaria Due to Immune Mechanisms

Urticaria following drug administration may occur without previous exposure to the drug or after previously tolerated exposures. Drugs appear to cause only a minority of cases of chronic urticaria, and while they are often assumed to be the cause of a high proportion of cases of acute urticaria during drug treatments, some results cast doubt on this. An examination, including skin testing, of 350 patients with suspected drug-induced reactions made up of 343 with urticaria/angioedema and seven with anaphylaxis revealed that only 22 (6.3%) were allergic and had a positive skin test to the suspected drug. The positive reactors proved to be the patients who presented with the most severe symptoms. An immediate (within 20 min) positive skin test is usually presumed to result from an IgE antibody-mediated mechanism or a direct histamine-releasing effect, but one cannot necessarily presume that these are the only mechanisms operative in all cases of drug-induced acute urticaria. Symptoms of urticaria are caused by the mediators histamine, leukotrienes, prostaglandin D2, bradykinin, and other vasoactive substances released from mast cells and basophils into the skin. Cases of acute urticaria may be immune- or non-immune-mediated. Drug-induced immune-mediated reactions can be elicited by cross-linking of high affinity (FcεRI)-bound IgE antibodies on mast cells and basophils by free drug or drug-carrier complex molecules reacting with the bivalent antibody combining sites via their complementary allergenic determinants. This results in degranulation of the cells and histamine release.

Other hypersensitivity responses may lead to urticarial reactions. A rare cause of the acute form is a type II hypersensitivity cytotoxic reaction mediated by cytotoxic antibodies and complement activation. An example of this type of reaction occurs in transfusion reactions when IgG and IgM antibodies activate complement and lyse transfused incompatible red cells. Urticaria may also result from a type III antigen-antibody complex-mediated hypersensitivity reaction, in particular, serum sickness lasting for several weeks and presenting with fever, arthralgias, and glomerulonephritis as well as urticaria. Note therefore that there is a drug-induced serum sickness-like reaction that is not associated with circulating immune complexes. Drugs implicated in these reactions include penicillins, cephalosporins, tetracyclines, quinolones, sulfonamides, NSAIDs, carbamazepine, thiouracil, allopurinol, and barbiturates. Other drug-induced type III hypersensitivity reactions involving skin inflammation include erythema nodosum leprosum induced by dapsone and the Jarisch-Herxheimer reaction following treatment of some microorganisms (e.g., in syphilis) with antimicrobials such as penicillins and tetracyclines. The inflammatory cytokines TNF, IL-6, and IL-8 appear to be released in these reactions. Urticarial vasculitis is another type III hypersensitivity skin eruption that can resemble urticaria and which is sometimes drug-induced. Antigen-antibody complexes formed in the vascular lumina lead to complement activation, chemotaxis of neutrophils, and the release of proteolytic enzymes that damage the vascular lumina. Drugs implicated include ACE inhibitors, penicillins, sulfonamides, thiazides, and the antidepressant fluoxetine. Urticarial reactions are also sometimes seen along with other skin manifestations during some drug-induced type IV hypersensitivity responses, but the presence of other skin manifestations, frequently more severe, makes it difficult to distinguish and study the specific mechanisms.

3.5.2 Urticaria with an Autoimmune Basis

A significant proportion of cases of chronic urticaria demonstrate no connection with drugs. Observations during the 1980s on the association of chronic urticaria and angioedema with thyroid autoimmunity and the prevalence of anti-IgE autoantibodies in urticarial syndromes suggested that autoimmunity might have a role in some cases of chronic urticaria. These findings led to the occasional demonstration of the presence of IgG anti-immunoglobulin E autoantibodies and functional autoantibodies against the
alpha subunit of the high affinity IgE receptor (i.e., FcεRIα) in at least one third of patients with chronic urticaria. These autoantibodies activate normal cell function by cross-linking the receptors on cutaneous mast cells and blood basophils, thus releasing histamine and other mediators responsible for the urticaria and angioedema. Activity of the autoantibodies was later shown to be augmented by complement activation with a critical role for component C5a. In some studies, high serum IgE levels have been found in up to 50% of patients with chronic urticaria, but compared to controls, no association with severity has been seen.

Chronic urticaria is now divided into autoimmune and idiopathic subgroups since in about 55–60% of patients the etiology remains obscure. As well as releasing histamine and leukotrienes from basophils, sera with the autoimmune antibodies also release IL-4. A study of lymphocytes from patients with chronic urticaria showed that activated CD4+ T cells produced high amounts of IL-4 and IFN-γ, strengthening the evidence for an immune basis of the disease and supporting histological demonstrations of predominant CD4+ T cell infiltrates in biopsies of chronic urticaria lesions. The observed cytokine profile of IL-4, IL-5, and IFN-γ does not reflect a predominance of Th1 or Th2 cells, and cellular infiltrates indicate a Th0 profile or a mixture of activated Th1 and Th2 cells.

Omalizumab, a recombinant humanized monoclonal antibody that inhibits the binding of human IgE to its high affinity receptor FcεRI by selectively binding the immunoglobulin in solution (Chap. 13), was originally approved as a successful treatment for intractable allergic asthma. The efficacy of the monoclonal antibody treatment was therefore investigated in patients with chronic autoimmune urticaria who remained symptomatic on antihistamine therapy. Of the 12 patients treated, 7 showed complete resolution of the urticaria, 4 responded with a decrease in the urticaria activity score but the urticaria persisted, and 1 patient showed no improvement. Omalizumab is currently the mainstay of treatment of antihistamine-resistant chronic spontaneous urticaria (see Sect. 3.5.5, Fig. 3.20, and Chap. 13, Table 13.2).

### 3.5.3 Basophils in Chronic Urticaria

CD203c is a basophil activation marker that is upregulated by cross-linking the FcεRI receptors on mast cells and basophils. Incubation of basophils with sera from patients with chronic idiopathic urticaria and a positive autologous serum skin test (ASST; an intradermal test with the patient’s own serum) demonstrated significant upregulation of CD203c, and this upregulation correlated with basophil histamine release and the ASST. Basophils from chronic urticaria patients are less responsive to anti-IgE and C5a but highly responsive when incubated with sera, even normal sera. The stimulatory factor(s) in serum has not been identified, and the increased response of the cells is not yet understood. In a flow cytometric evaluation of the expression of basophil cell surface markers CD203c, CD63, and CD123 and the receptor FcεRIα, CD203c and CD63 were both upregulated on basophils from patients with chronic idiopathic urticaria regardless of their ASST response. High expression of IL-3 receptor on basophils and activated T cells was detected only in ASST-positive patients.

### 3.5.4 Non-immune-Mediated Urticaria

Good evidence is often lacking for the mechanisms of what appear to be urticarias induced by apparent non-immunological events. Some suggested mechanisms include mast cell activation and “non-specific” mediator release through toll-like membrane receptors, receptors for complement, cytokines and chemokines, toxic xenobiotics including endogenous peptides, as well as somatostatin, substance P (induces histamine release through activation of MRGPRX2), endorphins, and enkephalins. Reactions are sometimes referred to as pseudoallergic responses since their clinical course and presentation are similar to allergic urticaria and angioedema. Implicated drugs, including the antimicrobial vancomycin (Chap. 6), neuromuscular blockers used in anesthesia (Chap. 7), opioid analgesics such as morphine (Chap. 8), NSAIDs (Chap. 9),
radiocontrast media (Chap. 10), and a wide range of other less often used medications, can trigger urticaria by directly stimulating mast cell degranulation and histamine release. The mechanism of mediator release by the NSAIDs is particularly interesting. The drugs inhibit cyclooxygenase which in turn leads to overproduction of the vasoactive and proinflammatory leukotrienes (Sect. 3.3.2 above). This subject is discussed in more detail in Chap. 9.

3.5.5 Drugs and Targets for Successful and Promising Treatments of Urticaria

Some drugs already approved for treatment of chronic urticaria or being used off-label, and drugs under development with promising treatment targets, can provide a good indication not only of likely future prospects for prevention and cure but also insights into the mechanisms underlying this heterogeneous disease. The present mainstays of treatment are H1 antihistamines with the licensed IgE-targeted monoclonal antibody (mAb) omalizumab for antihistamine-resistant patients, but several other promising therapies, including mAbs and tyrosine kinases, are being trialed. The mAbs ligelizumab and UB-221 show a 40–50- and eight-fold binding affinity, respectively, for IgE compared to omalizumab, and, in addition, UB-221 downregulates IgE synthesis by binding the antibody complexed to CD23. The already approved mAb dupilumab which binds the alpha subunit of the IL-4 receptor (IL-4Rα) thus modulating signaling of IL-4 and IL-13 and the approved mAbs mepolizumab and reslizumab, both targeted to IL-5, and benralizumab, targeted to the receptor IL-5Rα, have either been used or considered for off-label or beyond-label use. Both eosinophils and IL-5, which modulates eosinophils, have a well-known association with allergic
disease. A number of other approved mAbs and fusion proteins (adalimumab, infliximab, rituximab, abatacept, etanercept) indicated for autoimmune diseases are also receiving some consideration as potential treatments for urticaria (Fig. 3.20). Some additional promising potential treatment approaches include Bruton’s tyrosine kinase inhibitors (BTKs); Syk inhibitors; the targeting of MRGPRX2 and agonists such as substance P; antagonists of the histamine H4 receptor; antagonists for chemotactrant receptor-homologous molecule expressed on Th2 cells (CRTH2); mAbs to Siglec-8, a receptor expressed on mast cells, basophils, and eosinophils involved in asthma and allergy; inhibition of stem cell factor (SCF), the ligand of the mast cell receptor Kit and major activator of mast cells; and other novel approaches, many still at the level of the increasing understanding of mechanisms for controlling mast cell activation in patients with chronic spontaneous urticaria. Figure 3.20 is a comprehensive attempt to summarize the complex relationships between a number of new potential treatments.

### 3.6 Drug-Induced Angioedema

Angioedema (Chap. 2, Sect. 2.2.3) does not always have an allergic basis. Non-allergic angioedema not involving IgE antibodies and unassociated with urticaria can occur. The prototype example is hereditary angioedema arising from a deficiency of the inhibitor for C1 esterase that results in the maintenance of undegraded bradykinin. Acquired angioedema may be due to the accelerated consumption of C1 esterase inhibitor or, with an immune component, to autoantibody production. During acute attacks of hereditary and acquired angioedemas, plasma bradykinin has been shown to rise to up to 12 times the normal level.

#### 3.6.1 Angiotensin-Converting Enzyme (ACE) Inhibitors and Angioedema

ACE occurs as somatic and germinal isozymes. The somatic enzyme, expressed in the lungs and in vascular endothelial, kidney, and testicular Leydig cells, is part of the renin-angiotensin-aldosterone system, one of the body’s mechanisms for maintaining blood pressure. ACE has two actions – it catalyzes the conversion of the ten amino acid peptide angiotensin I to the potent vasoconstrictor eight amino acid peptide angiotensin II and degrades bradykinin, a potent vasodilator. The vasoconstrictor action of angiotensin II may lead to increased blood pressure and hypertension, the effect that led to the development and application of the ACE inhibitor drugs. ACE inhibitors increase bradykinin levels and prolong its action and decrease angiotensin II levels (and therefore a decrease in aldosterone secretion from the adrenal cortex) leading to dilation of blood vessels and a coincident decrease in arterial blood pressure. ACE inhibitors, now widely used to treat hypertension, congestive heart failure, and diabetic nephropathy, include the drugs benazepril, captopril, enalapril, fosinopril, lisinopril, perindopril, quinapril, ramipril, and trandolapril. The combination of actions of decreasing angiotensin II and aldosterone levels and increasing and maintaining bradykinin levels may lead to fluid extravasation into subcutaneous tissue ultimately producing angioedema. The increased levels of bradykinin are thought to be related to the high incidence of cough in patients on ACE inhibitors, and elevated bradykinin levels in the peripheral tissues, resulting in rapid fluid accumulation, are suspected of playing a key role in angioedema seen in a small number of patients taking ACE inhibitors. The association between ACE inhibitors and angioedema, first reported in the early 1980s, is now well recognized as a potentially serious but rare side effect of the drugs. Reactions occur with an incidence of about 0.1–0.5%, but the incidence in blacks (black Americans and Afro-Caribbean) is about three times higher than in white populations. This, and the decreased antihypertensive response to ACE inhibitions in blacks, is thought to be due to decreased production of bradykinin and/or decreased vasodilation in response to the peptide vasodilator. In terms of numbers and severity, ACE inhibitor-induced angioedema is said to account for 17% of patients admitted for the treatment of angioedema, and from 13 to 22% of
patients with this form of angioedema require airway intervention. In a 2008 study in Boston, USA, records of 220 patients who presented to five hospital emergency departments were reviewed. The frequency of ACE inhibitor-induced angioedema in all patients who presented with angioedema was 30%. The annual rate of visits for ACE inhibitor-induced reactions was 0.7 per 10,000 emergency department visits. Eleven percent of the patients were admitted to intensive care, and 18% admitted to hospital for observation for a 24 h period. This study confirmed past experience and surveys concluding that ACE inhibitor-induced angioedema remains a rare condition, it represents a significant proportion of angioedema patients, and a subgroup of these patients require hospitalization for management of upper airway angioedema.

Figure 3.21 summarizes the individual reactions and the interactions and relationship between the renin-angiotensin system and the plasma kallikrein-kinin system. Activation by the enzyme prolylcarboxypeptidase (lysosomal carboxypeptidase) of the prekallikrein high molecular weight kininogen complex on endothelial cells produces kallikrein which cleaves high (sometimes low) molecular weight kininogen liberating bradykinin. Bradykinin stimulates vasodilation and leads to the formation of nitric oxide (NO), superoxide, and prostacyclin and the liberation of tissue plasminogen activator. Kallikrein in plasma and tissues also activates prorenin to renin, an aspartyl protease, which in turn activates angiotensinogen to angiotensin I. ACE converts the inactive decapeptide angiotensin I to the biologically active octapeptide angiotensin II which, like bradykinin, stimulates NO and superoxide formation as well as contributing to the elevation of blood pressure and local vasoconstriction and stimulating the release of plasminogen activator inhibitor I. ACE is also a major degrading enzyme for bradykinin (in fact, bradykinin is its preferred substrate over angiotensin I) producing the breakdown pentapeptide bradykinin(1–5). In addition to its role in the formation of kallikrein, prolylcarboxypeptidase (with other enzymes) degrades angiotensin II to form angiotensin(1–7) which has vasodilatory and blood pressure-lowering activities. Overall then, stimulation of the bradykinin and angiotensin II receptors results in vasodilation and the production of NO and prostacyclin. Stimulation of the angiotensin I receptor leads to vasoconstriction and the elevation of blood pressure. It can be seen therefore that the kallikrein-kinin and renin-angiotensin systems interact and are linked in a mutually dependent way.

Although it is beyond our requirements here, it should be pointed out that a homolog of ACE, angiotensin converting enzyme 2 (ACE2), has recently been recognized. The two enzymes show different recognition of bradykinin. ACE2, a carboxypeptidase found mainly in the heart, kidney, and testis, does not degrade bradykinin but degrades des-Arg(9)-bradykinin at its carboxyterminal amino acid, and, unlike ACE which degrades angiotensin I by cleaving at the penultimate phenylalanine to produce angiotensin II [angiotensin(1–8)], ACE2 removes the carboxyterminal leucine to form angiotensin(1–9). This peptide has been reported to enhance arachidonic acid release.

### 3.6.2 Angioedema Following Administration of Angiotensin II Receptor Binding Inhibitors

Angiotensin II receptor blockers (ARBs) are primarily prescribed for high blood pressure but may also be used to treat heart attack, stroke, and congestive heart failure. Unlike ACE inhibitors, ARBs are not associated with cough. When first approved for the treatment of hypertension in 1995, ARBs were considered safe from the risk of edema, and they are generally a safe alternative to ACE inhibitors, blocking the renin-angiotensin system more effectively than the latter drugs. By binding selectively to the angiotensin 1 receptors (AT1), ARBs do not affect ACE and therefore should not affect bradykinin levels, but angioedema to ARBs does occur with an incidence ranging from about 0.1% to 0.4%. From limited numbers examined, the risk of
Fig. 3.21 Summary of the individual reactions involved in, and relationships between, the renin-angiotensin and plasma kallikrein-kinin systems. ACE, angiotensin converting enzyme; BP, blood pressure; MW, molecular weight; NO, nitric oxide.
patients with angioedema to ACE inhibitors developing angioedema to an ARB is said to be from 2% to 17% and, for developing confirmed angioedema, 0% to 9.2%. A review of 19 cases of ARB-induced angioedema found that 13 (68%) had never received an ACE inhibitor. Angioedema has been reported after administration of losartan, candesartan, eprosartan, irbesartan, olmesartan medoxomil, and telmisartan. Cross-reactivity between ACE inhibitor- and ARB inhibitor-induced angioedema has been estimated to be from 3% to 8%. Since angioedema to ACE inhibitors occurs as a result of increased bradykinin levels and ARBs are not known to affect these levels, the mechanism of ARB-induced angioedema is not understood. One suggested explanation is that unblocked angiotensin II receptors (AT2) are subjected to secondary stimulation by high levels of angiotensin II producing an increase in tissue bradykinin and hence angioedema. Another suggestion is an abnormality in the degradation of the active metabolite of bradykinin, des-Arg(9)-bradykinin.

3.7 The Allergen-Induced Late-Phase Reaction

Exposure to allergen in the skin, lung, nose, or eye of atopic patients provokes an immediate or early response that is maximal at 20–30 min, resolves within about an hour, and is often followed 3–4 h after allergen challenge by a delayed reaction peaking at 6–12 h and subsiding by 24 h. The two reaction phases are well illustrated by an asthmatic response in the lungs of an allergic patient measured as falls in the peak expiratory flow rate (PEFR) following inhalation challenge with allergen. Figure 3.22 shows that the immediate response bottoms at about 30 min after allergen challenge before beginning to recover and climbing back over the next 30 min toward, but not reaching, the pre-challenged PEFR figure. Three to four hours after the initial allergen challenge, there is a late-phase response reflected in a pronounced fall in PEFR which reaches its maximum at 5–10 h. Thereafter there is a steady climb back to normal levels. The immediate response is caused by the release of histamine and some other preformed mediators from mast cells that have direct effects on blood vessels and smooth muscle. The initial release of the preformed mediators is supplemented over time by other powerful inflammatory agents including vasoactive agents that dilate blood vessels and produce edema, swelling, and pain. Figure 3.23 shows good examples of immediate and late-phase cutaneous reactions. An immediate wheal and flare reaction and a late-phase edematous response are seen 15 min and 6 h, respectively, following intradermal injection of antigen.

**Fig. 3.22** A typical lung function result as measured by peak expiratory flow rates (PEFR) in an allergic patient following challenge with allergen. An immediate reaction at about 30 minutes is followed by a late-phase response which reaches a maximum 5 to 10 h after allergen challenge.
3.7  The Allergen-Induced Late-Phase Reaction

3.7.1 Early Studies: Implication of IgE Antibodies

Late reactions are well-known with initial published reports dating back nearly 100 years, but investigations of the underlying cellular events and mechanisms involved were not pursued in any systematic way until the late 1960s when Pepys and colleagues studied patients with allergic bronchopulmonary aspergillosis and extrinsic allergic alveolitis, also known as hypersensitivity pneumonitis. They found edema, perivascular cellular infiltration, deposition of complement and serum immunoglobulin precipitins to \textit{Aspergillus fumigatus}, and a variety of other extracts from agents that cause allergic alveolitis and concluded that the late reactions were the result of an Arthus or type III reaction. Soon after, other investigators came to a different conclusion failing to consistently find precipitins and complement but strongly implicating IgE antibodies in the reactions by a variety of methods including direct demonstration by induction of immediate and late responses with affinity-purified allergen-specific IgE antibodies followed by allergenic challenge. Another important finding was the observation that lymphocytes were the predominant cell in the cellular infiltrates together with a significant number of eosinophils and basophils. It should be remembered, however, that the investigations implicating IgE antibodies in late reactions to \textit{Bacillus subtilis} enzyme, ragweed pollen, and other inhalant allergens do not necessarily refute the conclusion of a type III, Arthus reaction to \textit{Aspergillus} species and other allergens responsible for hypersensitivity pneumonitis conditions such as bagassosis and farmer’s, bird-fancier’s, coffee worker’s, malt worker’s, and mushroom worker’s lung. These conditions are very different to hypersensitivities to allergen sources such as ragweed pollen and are characterized by different antigenic stimuli, symptoms of cough, dyspnea, pleurisy, fatigue, anorexia, and weight loss with interstitial granulomas and mononuclear and giant cells in the lungs.

3.7.2 Cellular Responses in the Late-Phase Reaction and Comparison with the Delayed-Type Hypersensitivity Response

From undergraduates to clinicians and researchers, there has long been confusion over use of the terms “late” and “delayed” with the late phase of the immediate wheal and flare reaction sometimes being labeled and referred to as a delayed-type hypersensitivity reaction, a type IV reaction, or simply “DTH.” There was therefore a need to research, compare, and contrast these reactions, and this was done in an important study in which both responses were provoked in the same individuals and studied with the same panel of cell marker monoclonal antibodies together with immunohistologic methods. Skin biopsies from atopic individuals with late-phase allergic skin reactions to intradermal challenge with grass
pollen or house dust mite were sectioned and examined for evidence of infiltration and activation of T cells and eosinophils. A substantial number of CD3+ and CD4+ cells but far fewer CD8+ cells were observed together with clearly different CD4+:CD8+ ratios in the sampled tissue and the peripheral blood. Infiltrated cells bearing receptors for IL-2 and evidence for IFN-γ secretion suggested that T cells had become activated. Activated eosinophils were also detected, and there was a strong correlation between these cells and the numbers of CD4+ cells 24 h after the allergen challenge suggesting that T cells participate in the late-phase inflammatory reaction. In fact, about 50% of cells infiltrating a late-phase reaction site are T lymphocytes. It is therefore of interest to compare the late-phase response with the delayed-type hypersensitivity reaction since it seems that T lymphocytes are important in both responses. In the comparison, grass pollen and house dust mite extracts were used to elicit late-phase reactions, while tuberculin challenge was used for delayed hypersensitivity responses. Both responses showed accumulation of CD4+ T cells, but overall, the cells were more dispersed in denser accumulations and cells were still being recruited at 48 h in the delayed reactions. This contrasts with the situation in late-phase reactions where cell numbers usually plateau between 24 and 48 h. Other differences found were greater activation of eosinophils in late-phase reactions, the detection of small numbers of these cells in atopics and nonatopics at 24 h (but not at 48 h) in delayed-type hypersensitivity, and greater T cell activation (demonstrated by expression of IL-2R) in the latter response. The release of inflammatory cytokines in both reactions was indicated by endothelial expression of HLA-DR. The allergen-induced late-phase reaction then has features of a cell-mediated hypersensitivity response, but it shows some significant differences from the classical delayed hypersensitivity response in atopic subjects. The difference is perhaps best illustrated by the different cytokine profiles. Employment of labeled RNA probes for some cytokines showed that infiltrating cells from allergen-induced late-phase cutaneous reactions have a Th2-like cytokine profile expressing mRNA for the cytokine gene cluster IL-3, IL-4, and IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF). Cells from tuberculin biopsies on the other hand preferentially expressed mRNAs encoding for IL-2 and IFN-γ, that is, cells preferentially expressing a Th1 cytokine profile. Comparisons of the accumulation of inflammatory cells and cells expressing mRNA for different cytokines in late-phase and delayed-type responses in the same subjects showed a relatively rapid (1–6 h) accumulation of T cells and granulocytes in the former case and a much longer accumulation time (24–48 h) for T cells, macrophages, and other cells expressing Th1-type cytokines in delayed hypersensitivity responses. At 48–96 h in the late-phase response, some cells increasingly expressed Th1-type cytokines. This may be an indication of a classic delayed hypersensitivity response earlier masked by the IgE antibody-mediated reaction. Again, with the delayed-type response, the distinction from the late-phase response was not totally clear since a small number of cells in some individuals expressed mRNA encoding IL-4 and IL-5.

3.8 Delayed-Type (Type IV) Hypersensitivity Drug Reactions

Unlike types I, II, and III hypersensitivities, which are mediated by antibodies, delayed or cell-mediated hypersensitivity, classified as type IV, is mediated by antigen-specific effector T cells, and this means that the hypersensitivity response can be transferred by purified T cells or a cloned T cell line. Again, in contrast to an immediate reaction, a delayed-type hypersensitivity reaction develops over a period of 24–72 h. Delayed hypersensitivity responses have been used for many years to assess patients’ cell-mediated immunity by the induction of induration and erythema 48–72 h after intradermal injection of so-called “recall” antigens from
Mycobacterium tuberculosis, Candida, and Trichophyton species and tetanus toxoid. For the first episode, delayed cutaneous adverse drug reactions generally begin 7–21 days after contact with drug. Subsequent reactions begin 1 or 2 days after re-exposure. Specificity is usually demonstrated by oral challenge with small doses of the culprit drug, a positive patch test, or intradermal test generally read after a delay of at least 48 h and perhaps a positive in vitro lymphocyte proliferation assay. Activated T cells are found in the skin, and in some cases T cell lines and clones can be isolated from blood and/or skin sites. Distinct subsets of T cells with their accompanying profile of cytokines and chemokines promote the inflammatory and cytotoxic responses seen in the different clinical patterns characteristic of the various drug-induced adverse cutaneous hypersensitivities. Individual hypersensitivity eruptions are essentially the result of overlapping cytokine actions with one or a few such actions dominant and characteristic of the delayed drug hypersensitivity phenotypic pattern. This, and the lack of histological and immunocytochemical criteria, has consequences for the diagnosis of drug-induced skin reactions where considerable effort is needed in the development of reliable and specific tests that can be easily undertaken (see Chap. 4). Although the mechanisms underlying the different drug-related skin eruptions with an immunological pathogenesis are still far from precisely defined, summaries of the progress are set out below.

### 3.8.1 The Cellular Basis of Type IV Hypersensitivity Cutaneous Drug Reactions

Delayed-type hypersensitivity reactions in the skin provoked by systemic drug administration usually occur 7–10 days after the commencement of therapy. Drug-induced skin reactions manifest mainly as exanthemas, mediated by CD4+ and CD8+ CD3+ T cells in the dermis and epidermis. Antimicrobial drugs, non-steroidal anti-inflammatorys, and some analgesic drugs are the biggest causes of drug-induced adverse cutaneous reactions, but a variety of other drugs including anticonvulsants (e.g., carbamazepine), local anesthetics (lidocaine), cardiovascular drugs (procainamide), and antipsychotics (clozapine) are well-known to cause reactions. For most proteins and hapten-protein conjugates, processed antigen is presented to CD4+ T cells via the MHC class II molecules on antigen-presenting cells. The cells involved in many type IV responses such as contact hypersensitivity and psoriasis are Th1 and CD8+ cytotoxic T cells, while in a condition such as allergic contact dermatitis, CD4+ or CD8+ T cells can be activated depending on the antigen processing pathway. In general, CD4+ T cell activation seems to mediate maculopapular and eczematous drug hypersensitivities, while CD8+ T cell activation produces the more severe skin reactions involving bullous manifestation.

In a hypersensitivity reaction in the skin such as allergic contact dermatitis, there are two phases of the hypersensitivity response, sensitization (or initiation or induction) and elicitation. In the sensitization phase following drug administration, free drug or drug bound to a protein carrier reaches the skin where it encounters keratinocytes present in great numbers and thought to play a major role in the initiation of skin sensitization. As well as facilitating the formation of biologically active haptens and hapten binding to protein, keratinocytes release chemotactic factors CXCL8, CXCL9, CXCL10, and CXCL11 and adhesion molecules (e.g., ICAM-1) on exposure to sensitizing agents. These chemotactic factors attract more cells to the active skin sites, thus increasing local immune activity. Sensitization proceeds with drug-carrier complex being taken up by immature Langerhans and dendritic cells. These migrate to the draining lymph node and, with the stimulus provided by co-stimulatory molecules, become T cell-activating cells. Processed antigen is expressed as a drug-peptide complex in association with MHC class I and II molecules on the surface of the
mature antigen-presenting cells for presentation to CD8+ and CD4+ T cells, respectively. Dendritic cells, Langerhans cells, and skin macrophages express both MHC (class I and II) molecules and can activate CD4+ as well as CD8+ T cells. Keratinocytes are also important in the elicitation phase and can present antigen via MHC I and MHC II molecules. T cells are activated, undergo clonal expansion, and give rise to cells with different memory and effector functions, that is, Th1, Th2, or Th17 CD4+ or CD8+ T cells. By the time of the elicitation phase, T cells have gained access to the skin, and following re-exposure to the drug, skin symptoms occur within about 48 h. Hapten-specific T cells recognize the hapten-peptide presented by dendritic cells and keratinocytes, and the resultant activated T cells produce Th1 and Th17 cytokines such as IFN-γ, IL-12, IL-17, and IL-23. Note that although Langerhans cells have long been considered to be the classical cell net to trap, process, transport, and present antigen to T lymphocytes, evidence, including mice lacking Langerhans cells, has shown that dendritic cells can act in their place if Langerhans cells are absent or functionally affected. The nature of the antigen, that is, the sensitizing drug or drug conjugate, seems to determine which MHC molecule is involved in antigen presentation. Although extracellular antigens (e.g., contact allergens) are generally presented via MHC class II molecules and intracellular antigens (e.g., drug-protein conjugates formed intracellularly) via MHC class I, presentation of some extracellular contact sensitizing agents, for example, nitrobenzene sulfonic acid, appears to be by MHC II molecules.

With the involvement of the co-stimulatory B7-CD28 interaction, T cells are activated, and memory T cells can be found in the dermis. Other co-stimulatory molecules have also been identified including OX-40-OX-40 L, PD-1-PD-L1 and PD-L2, RANK-RANKL, and CD40-CD40L (CD154). The receptor OX-40, also known as CD134, and its ligand OX-40 L are seen as secondary co-stimulatory molecules expressed after T cell activation and important in maintaining T cell memory. RANKL, involved with dendritic cell maturation, belongs to the TNF cytokine family, while PD-1 and its ligands, belonging to the B7 family, negatively regulate T cell responses. Binding of the co-stimulatory molecule CD-40 on antigen-presenting cells to its ligand CD40L activates these cells. During subsequent exposure of the memory T cells to the sensitizing antigen, clonal expansion of the activated T cells occurs, and this ultimately results in T cell-mediated inflammation and cell damage in the skin. Activation and proliferation of memory T cells in the dermis release chemokines and inflammatory cytokines such as IFN-γ and tumor necrosis factor-α/β (hereafter referred to as TNF) that recruit macrophages to the site. Presentation of antigen by the newly arrived macrophages has the effect of further amplifying the response. The released chemokines and cytokines increase the permeability of blood vessels leading to local swelling and induce the expression of vascular adhesion molecules. IFN-γ is the key cytokine, and it plays a dominant part in delayed hypersensitivity, upregulating T cell activation markers and MHC molecules and aiding Th1 while suppressing Th2 cell differentiation. TNF also has a central role in delayed hypersensitivity, inducing chemokine production, upregulating expression of adhesion molecules, and promoting the influx of inflammatory cells. CD4+ and CD8+ T cell-mediated cytoxicity of skin cells presenting drug can result from interaction with Fas/FasL, release of the cytolytic protein perforin and the serine protease granzyme B from cytotoxic T lymphocytes, and release of granulysin from cytotoxic CD8+ T cells. In response to inflammatory agents released by T cells, skin cells can in turn contribute to inflammation by releasing their own spectrum of cytokines and chemokines that stimulate further leukocyte recruitment into the skin. As understanding of the complexities of the mechanisms of the many processes that make up delayed-type hypersensitivity responses increases, two other agents, IL-12 and osteopontin, are attracting the interest of researchers. IL-12, produced mainly by antigen-presenting cells, aids the proliferation and differentiation of
Th1 cells, augments IFN-\(\gamma\) production by these cells, and enhances NK and CD8+ T cell cytotoxicity. Osteopontin (also known as ETA, early T lymphocyte activation-1), a phosphoglycoprotein with cytokine and chemotactic functions, has a Th2 suppressive effect augmenting Th1-mediated allergy such as allergic contact dermatitis and supporting dendritic cell migration and IL-12 expression and secretion. Discussion of osteopontin’s role in allergic contact dermatitis is continued briefly below in Sect. 3.8.3.

### 3.8.2 T Helper Cell Responses and Th17

As discussed, naïve T cells differentiate into Th1 or Th2 cells during activation induced by interaction with dendritic cells with toll-like pattern-recognition receptors that detect the nature of the antigen. This results in IL-12 production; the involvement of transcription factors T-bet, STAT4, or STAT1 within the T cell; the induction of Th1 differentiation; and production of IFN-\(\gamma\). Th2 differentiation is the result of IL-4 cytokine and GATA-3 and STAT6 transcription factor involvement that drives production of IL-4, IL-5, and IL-13. TGF-\(\beta\) and the transcription factor FoxP3 results in the Treg T cell subset that secretes TGF-\(\beta\) (Fig. 3.24). After definition of the Th1 and Th2 subsets more than 20 years ago, relatively recent research has revealed a new class of T effector cells Th17, induced from naïve T cells by the cytokines TGF-\(\beta\) and IL-6 and enhanced by IL-23, a cytokine produced by keratinocytes, Langerhans cells, dendritic cells, and macrophages.

**Fig. 3.24** Naive T cells, under the influence of cytokines produced by other immune cells, undergo activation and polarization to distinct Th subsets. Each subset displays a distinct cytokine secretion profile resulting in different effector functions, e.g., Th1 cells activate macrophages; Th2 cells promote allergic responses and immune responses to parasites; Th17 cells promote inflammation by helping to recruit neutrophils; and Treg cells exert a number of inhibitory actions via cell contact. A more recently identified CD4 T cell subset, termed follicular helper cells (Tfh), provide a helper function to B cells.
phages. Th17 cells are characterized by expression of distinct transcription factors RORγT, STAT3, and IRF-4 and the production of proinflammatory cytokines IL-6 and TNF and chemokines CCL2 (monocyte chemotactic protein-1 or MCP-1), CXCL1, and CXCL2 that activate macrophages and granulocytes. IL-25 and IL-27 negatively regulate Th17 cells, while Th17 polarization is inhibited by IL-2, IL-4 (induces Th2), and IFN-γ (induces Th1).

Another more recently identified CD4 T cell subset, termed follicular helper cells (Tfh) (Fig. 3.24), provides a helper function to B cells. Tfh cells are distinguished from Th1 and Th2 cells by expression of the chemokine CXCR5, their association with B cell follicles, and their B cell helper function. They produce ICOS (inducible T cell co-stimulator) and IL-21, a cytokine that stimulates B cells to differentiate into antibody-forming cells. This cytokine is particularly interesting for those concerned with understanding allergic mechanisms and the treatment of immediate-type allergies. IL-21 knock-out mice express higher levels of IgE than normal mice, and, in fact, IL-21 has already been used to attenuate allergic responses by reducing both IgE and inflammatory cytokine production in mouse models for rhinitis and peanut allergy.

3.8.3 Allergic Contact Dermatitis

For a description of allergic contact dermatitis, see Chap. 2, Sect. 2.5.1 and Figs. 2.6, 2.7, 2.8. Not all contact dermatitis has an immune basis; some irritants such as organic solvents, highly alkaline drain cleaners, and sodium lauryl sulfate and some photoxins like the psoralsens, paradoxically used for the treatment of psoriasis, eczema, and vitiligo, may also provoke reactions. Common causes of allergic contact dermatitis include nickel metal (Ni), chromium, balsam of Peru, and Toxicodendron plants, for example, poison ivy, poison oak, and poison sumac. Causative agents tend to be reactive small molecules or haptens of less than 1000 Da that can easily penetrate the skin barrier and form covalent adducts with cutaneous proteins. Allergic contact dermatitis is regarded as a Th1 and CD8+ T cell-mediated disease, and Ni allergy (Fig. 2.5, Sect. 3.9.1), which involves activation of HLA-restricted, skin-homing Ni-specific T cells by antigen-presenting cells, is perhaps its best-known commonly occurring form. Both sensitization and skin reactions to Ni are thought to be mediated by CD4+ and CD8+ effector T cells producing IFN-γ. During sensitization when no clinical symptoms are apparent, mature Langerhans cells originating from skin sub-layers present Ni-peptide-MHC complex to T cells in local lymph nodes. Upon rechallenge with Ni, the effector phase of allergic contact dermatitis is activated to produce cutaneous infiltration of Ni-specific and CCR4-positive T cells. Ni-specific cytotoxic CD8+ T cells release inflammatory cytokines that produce the characteristic skin lesions at the site of Ni sensitization (Chap. 2, Fig. 2.5). The T cell cytokine IL-17 can be found in the skin of patients with allergic contact dermatitis. Some Ni-specific CD4+ T cell clones isolated from the blood of allergic contact dermatitis patients express this cytokine which regulates the expression of adhesion molecules by keratinocytes and the synthesis and release of the chemokines IL-8 and RANTES. IL-17 has been shown to be locally released by Ni-specific Th0, Th1, and Th2 lymphocytes in the skin of patients with allergic contact dermatitis where it amplifies reactions and modulates the proinflammatory action of keratinocytes by acting together with IFN-γ and IL-4. There still seems much to learn about the role of IL-17 in allergic contact dermatitis, but already the importance of this cytokine in the pathomechanism underlying the condition is apparent.

Biopsies following a contact dermatitis reaction to Ni showed infiltration of CD4+ and CD8+ T cells in the skin with reduced CD3+, CD4+, and CD8+ cells in peripheral blood of patients.
previously sensitized to nickel and subsequently challenged with it orally. Elevated levels of the cytokines IL-2 and IL-5 were found in patients with dermatitis to nickel and elevated TNF and IL-1 in patients previously sensitized to gold.

The phosphoglycoprotein osteopontin is expressed by a number of different immune cells including effector T cells and keratinocytes in allergic contact dermatitis. The molecule is expressed in secreted and intracellular form; it enhances Th1 and Th17 immunity and protects against apoptosis. Experiments in mice have shown that T cell clones secreting low levels of IFN-γ may compensate by secreting high levels of osteopontin which, in turn, down-modulates T cell IL-4 expression. In allergic contact dermatitis, secretion of IFN-γ by effector T cells induces osteopontin in keratinocytes which ultimately results in the attraction of inflammatory cells. The demonstrations that osteopontin-null mice display a reduced inflammatory response in contact hypersensitivity and anti-osteopontin antibodies partly suppress established chronic contact sensitivity suggest that osteopontin may be a promising therapeutic target in allergic contact dermatitis.

### 3.8.4 Maculopapular Exanthema

A case of maculopapular exanthema induced by amoxicillin with lesions on the trunk and hands is shown in Fig. 2.9. For a clinical description see Chap. 2, Sect. 2.5.2. Lymphocytes (CLA+, CD3+, DR+, CD25+) expressing adhesion molecules are attracted from the blood by adhesion molecules expressed by endothelial cells and keratinocytes and by chemokines such as CCL27 (also called cutaneous T cell-attracting chemokine CTACK). Both CD4+ and CD8+ T cells are found in the skin and blood of patients with maculopapular exanthema, but findings on the relative importance of these cells differ with some authors stating that CD4+ cells predominate at the dermo-epidermal junction zone or in the epidermis and inflict cell damage by expressing high levels of perforin and granzyme B while CD8+ cells are found mainly in the epidermis. Perforin-positive T cells have been found in patients and CD4+ T cells eluted from patch tests after drug-induced reactions. Other results have shown that CD8+ cells predominate in acute lesions of the epidermis and are the major drug-specific cytotoxic cell found in the blood of most patients with penicillin-induced maculopapular exanthema. Activation of CD8+ T cells by drugs, an MHC class I mechanism, can sometimes lead to severe bullous exanthems. Examination of cellular infiltration in the skin of patients during patch testing demonstrated rapid recruitment of CD8+ cells after skin contact with drug and before appearance of other cells particularly CD4+ T cells which express perforin in the acute phase. Both type I and type II cytokines are produced; IFN-γ (type I) activates dendritic cells and keratinocytes; IL-5 (type II) together with eotaxin (CCL11) recruits and activates eosinophils. Other chemokines, including CCL20, CXCL9, and CXCL10, appear to be involved in skin homing.

### 3.8.5 Fixed Drug Eruption

For a clinical description of FDE, see Chap. 2, Sect. 2.5.3 and Figs. 2.10 and 2.11. Mediated by activated CD8+ T cells, fixed drug eruption is a disease instigated by drugs in more than 95% of cases. In regression, large numbers of CD8+ effector memory T cells of phenotype CD3+, CD45RA+, CD11β+, and TCR-αβ are found in lesions in the epidermis. Re-exposure to the culprit drug rapidly leads to a conversion of this benign state to one of aggressive cell damages. CD8+ T cells activated via the CD3-TCR complex secrete IFN-γ in high amount as well as TNF, perforin, granzyme B, and Fas ligand (FasL) which initiates cell killing by binding to its receptor FasR on keratinocytes. The presence of the “dormant” CD8+ T cells in “resting” lesions explains why patch testing is negative on normal skin, but reactivation occurs when patches are applied to areas of residual lesions.
3.8.6 Symmetrical Drug-Related Intertriginous and Flexural Exanthema (SDRIFE; Baboon Syndrome)

For a clinical description of SDRIFE, see Chap. 2, Sect. 2.5.4 and Fig. 2.12. There are indications, particularly histological features, that SDRIFE is more heterogeneous than originally described. In an early study induced by intravenous immunoglobulins, immunohistological investigations by Barbaud and colleagues showed that most perivascular T cells were CD4+ and endothelial cells exhibited CD54 (ICAM-1), CD26E (E-selectin), and CD62 (P-selectin, GMP). Keratinocytes also unexpectedly expressed CD62P which the investigators suggested might favor T cell recruitment to the skin and be involved in the still poorly defined mechanisms underlying the development of SDRIFE. Histology of a positive skin test to iodinated contrast media in a patient with SDRIFE revealed infiltration of the dermis with mainly CD3+ and CD4+ cells and a few CD8+, CD20, and CD56 cells, findings compatible with a T cell-mediated hypersensitivity.

The reason for the occurrence of the cutaneous eruptions of SDRIFE in the intertriginous and flexural regions is not known. One suggestion is that the condition is a recall phenomenon of any form of dermatitis, drug-induced or not, while another theory proposes the excretion of drugs or their metabolites from the high density of eccrine glands in the commonly affected areas.

3.8.7 Psoriasis

Clinical aspects of psoriasis are presented in Chap. 2, Sect. 2.5.5 and Figs. 2.13, 2.14, 2.15, and 2.16. The classification of T cells into Th1 and Th2 cells, essentially on the basis of their defining cytokines IFN-γ and IL-4, respectively, and the identification of the Th17 T cell, together with the realization of its importance in inflammation, have led to the re-examination of many diseases previously considered to be solely Th1- or Th2-mediated. So far, in murine models at least, some diseases previously thought to be Th1-mediated responses have been found to involve both Th1 and Th17 cells. Th17 cells produce IL-17, TNF, IL-6, IL-21, and IL-22 which is upregulated during inflammatory disorders and which produces thickening of mouse epidermis suggesting a role in psoriatic inflammation. Other findings suggestive of a role for Th17 cells in psoriasis include the reduction of levels of IL-17 and IL-22 in the serum of patients whose psoriasis had been cleared by treatment with the TNF inhibitor etanercept; enhanced expression of IL-23 in patients with psoriatic lesions; and the demonstration of IL-17 mRNA in psoriatic lesions. Analysis of psoriatic skin lesions and peripheral blood for the presence of IL-17-producing cells revealed Th17 cells localized in the lesions and the dermis. In addition, IL-17 mRNA expression returned to normal with cyclosporin therapy, and IL-22 mRNA expression moved in parallel with IL-17 changes suggesting that both Th1 and Th17 cells are active in the inflammatory stages of psoriasis. Following the demonstration that in addition to Th1 cells producing IFN-γ, CD4+ T lymphocytes producing IL-17 were also important in the pathogenesis of psoriasis. Attention then turned to the possible importance of IL-17-producing CD8+ cells known to be present in psoriatic plaque. Investigations showed that CD8+ IL-17+ cells produced the Th1-related cytokines IFN-γ and TNF as well as the Th17 cytokines Il-17, IL-21, and IL-22 and upregulation of the transcription factor RORC. These results showing some common properties between CD8+ IL-17+ T cells and Th17 cells and the intriguing finding that CD8+ cells, unlike Th17 cells, can also make IFN-γ and TNF may prove significant in fully elucidating the pathogenesis of psoriasis.

The current broad understanding of the events and mechanisms leading to psoriasis is summarized here as four phases. In the first phase (Fig. 3.25), keratinocytes activate den-
Dendritic cells in the dermis via the production of IL-1β, IL-6, TNF, and the cathelicidin antimicrobial peptide LL37/self RNA. **Phase 2:** By presenting antigen and secreting cytokines IL-1β, IL-6, IL-12, IL-23, and TNF, activated dendritic cells stimulate differentiation and activation of T cells producing IL-17. **Phase 3:** At the third phase, activated T cells secrete cytokines IL-17A, IL-17F, IL-22, and IFN-γ that activate keratinocytes (phase 4), inducing aberrant differentiation with the production of proinflammatory mediators including chemokines (CXCL1, CXCL2, CXCL5, CXCL8, CLCX9, CLCX10, CCL20) that recruit other immune cells, in particular neutrophils and T cells. Antimicrobial peptides are also produced.

### 3.8.8 Acute Generalized Exanthematous Pustulosis

Clinical aspects of acute generalized exanthematous pustulosis (AGEP) are presented in Chap. 2, Sect. 2.5.6 and Fig. 2.17. Activated drug-specific CD4+ and CD8+ T cells producing the neutrophil-attracting chemokine CXCL8 (IL-8) infiltrate the skin of patients with AGEP and can be detected in peripheral blood, positive patch test biopsies, and T cell lines and clones. CXCL8-producing effector memory T cells express mainly IFN-γ, GM-CSF, TNF, and sometimes IL-4 and IL-5. These cells express the chemokine CCR6 and aid infiltration and
survival of neutrophils leading to the sterile pustular eruptions found in AGEP patients (Fig. 2.17). Stepwise putative pathogenic mechanisms in AGEP are shown in Fig. 3.26. Drug-activated dendritic cells lead to activation and expansion of drug-specific CD4 and CD8 T cells (Fig. 3.26a) which migrate to the skin (Fig. 3.26b) where (perhaps with other killer cells) they cause apoptosis of keratinocytes and formation of subcorneal vesicles. This cell death is mediated by the cytotoxic proteins granzyme B, perforin, and granulysin and Fas/Fas ligand (FasL). CXCL8, thought to promote pustule formation by the recruitment of neutrophils, is released along with IL-17, IL-22, and GM-CSF (Fig. 3.26c). Mutations in the IL-36RN gene that encodes the IL-36 receptor antagonist (IL-36Ra) appear to correlate with the development of drug-induced AGEP, for example, a deficiency in IL-36Ra seems to be associated with increased expression of proinflammatory cytokines and chemokines including IL-1, IL-6, IL-12, IL-17, IL-23, TNF, and CXCL8 (Fig. 3.26d). This leads to further recruitment of neutrophils, increased inflammation, and pustule formation (Fig. 3.26c–e).

3.8.9 Drug Reaction (Rash) with Eosinophilia and Systemic Symptoms

The pathophysiology of drug reaction (sometimes designated rash) with eosinophilia and systemic symptoms (DRESS), also called drug-induced hypersensitivity syndrome (DIHS), is still being worked out (see also Chap. 2, Sect. 2.5.7; Figs. 2.18, 2.19). Some HLA variants are known to be associated with drug-induced DRESS in certain populations, for example, carbamazepine with HLA-B*1502 in Southeast Asian populations and with HLA-B*3101 in European populations. Early work indicated that activated CD4+ and CD8+ T cells expressing CCR10 and producing type I cytokines, chiefly IFN-γ, are found in the blood of DRESS patients.
in the acute phase, and these cells increase in proportion to the severity of the skin reaction. T cell clones from carbamazepine- and lamotrigine-sensitive patients were reported to react specifically with antigen-presenting cells apparently without the formation of reactive metabolites and processing, much like the situation with a superantigen. T cell clones from DRESS patients were also claimed to produce perforin and secrete IFN-γ and IL-5, the last of these accounting for the eosinophilia associated with the syndrome. Many investigators believe that a concomitant human herpes virus 6 (HHV-6) reactivation with hypogammaglobulinemia caused by the drug is associated with the hypersensitivity syndrome. Reactivation of HHV-6 may not be essential in the development of DRESS/DIHS, but it might be a factor in the severity of the syndrome and it
might affect its course. This remains to be clearly established, but the virus is associated with fever and skin rash and large anti-herpes virus and anti-EBV T cell responses have been detected in DRESS patients along with systemic inflammation associated with organ failure. Activated T cells produce TNF, IFNγ, and IL-2, mediators that may produce the symptoms seen in DRESS patients. Note that reactivation of, and infection with, herpes virus is not proven in Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Immunohistochemical examinations of skin biopsies and patch test samples from a patient with ceftriaxone-induced DRESS showed positive results for perforin, granzyme B, FasL, IFNγ, and IL-5.

In a study of the CD8+ T cell repertoire with the clinical severity of DRESS, fluctuation in the repertoire during the course of the disease positively correlated with the severity of symptoms and the anti-EBV immune response. CD8+ cells secreting TNF, IFNγ, and IL-2 were seen in patients with severe cutaneous symptoms, and these occurred in greater number than in patients with mild symptoms. Results suggested that continued production of CD8+ T cell clones may be responsible for sustaining secondary tissue injury, and fluctuations in the CD8+ repertoire suggested that newly formed clones may also have a role in the maintenance of DRESS injury. The authors concluded that although detailed mechanisms underlying the initiation and development of DRESS remain unclear, the fluctuating CD8+ T cell repertoire supports the conclusion that “herpesvirus-mediated spreading reactivation is an alternate mechanism responsible for the pathogenicity of DRESS.”

Although the “E” in DRESS is for eosinophilia, surprisingly few studies seem to have been directed to the role of this cell in the syndrome. Eosinophil infiltration is pronounced in DRESS, being found in 80% of diagnosed patients. IL-5, a key cytokine for eosinophil recruitment, proliferation, and activation, along with eotaxin-1 (CCL11) and thymus activation-regulated chemokine (TARC, CCL17), acts to recruit eosinophils in drug-induced cutaneous eruptions as seen in DRESS. Eosinophils infiltrate organs and release cytotoxic granule proteins, an important contributor to tissue damage in DRESS patients.

**Drug-Induced Liver Injury (DILI)** While mentioning DRESS, it is opportune to comment on drug-induced allergic hepatitis. As in DRESS, this condition is associated with fever, rash, eosinophilia, and liver infiltrates, and the reaction is generally a type IV hypersensitivity response involving CD4+ cells, CD8+ cytotoxic lymphocytes, NK, Kupffer, and dendritic cells. Type II hypersensitivities may also sometimes occur. There are two main hypotheses, for the mechanism of DILI caused by immune processes. First, the drug or active metabolite(s) acts as a hapten and binds to endogenous proteins forming conjugates that induce antibody- and/or T cell-mediated injury. Proponents of the second hypothesis suggest that most individuals are tolerant to immune-mediated DILI and reactions occur only when tolerance is overcome. Although the cellular events remain poorly defined, knowledge of underlying mechanisms of idiosyncratic DILI is even more fragmentary.

### 3.8.10 Drug-Induced Erythema Multiforme

For a description of erythema multiforme, see Chap. 2, Sect. 2.5.8, Figs. 2.20, 2.21. Erythema multiforme is a self-limiting cutaneous eruption that occurs most often after infections, in particular herpes simplex virus infection. Only a small number of cases are induced by drugs, and these appear to have a distinct pathogenesis compared to the reactions induced by infections. Histologically, the drug-induced condition shows less inflammation and increased necrosis of keratinocytes but few if any T cells, unlike the herpes virus-associated reactions involving CD4 T cells. The epidermal lesions in the drug-induced condition appear to be caused by perforin and granzyme B together with TNF produced by macrophages and monocytes not Th1 cells as occurs in the virus-associated form of the eruption.
3.8.11 Toxic Epidermal Necrolysis and Stevens-Johnson Syndrome

These diseases (Chap. 2, Sect. 2.5.9) are provoked by drugs in more than 90% of cases with sulfonamides, anticonvulsants, some NSAIDs, and allopurinol most frequently involved. It is not yet fully understood why and how a cutaneous adverse drug reaction very occasionally progresses to the life-threatening TEN or SJS. Clinical features of both syndromes are similar with the extent of necrotic epidermis/skin detachment greater in TEN (>30%; Figs. 2.22, 2.23) than in SJS (<10%) (Fig. 2.24) and the predominance of lesions around mucosal orifices in SJS (Fig. 2.25). In fact, the two disorders are considered by many to be variants of the same disease with different severity. In TEN, blister fluid contains many activated HLA class I-restricted, drug-specific CD8 + CD56+ cytotoxic T cells with natural killer (NK) cell features. These kill lymphocytes and particularly keratinocytes via, according to different researchers, several mechanisms including the Fas/FasL, TNF, granzyme B, perforin, TWEAK (TNF-like weak inducer of apoptosis), and TRAIL (TNF-related apoptosis-inducing ligand) pathways. These cytotoxic mediators are found in the serum as well as in blister fluid where levels are high and where they occur with other cytokines including IFN-γ, IL-10, and IL-18. Several studies suggested that TNF has an important role in TEN, and this appears to be supported by the success of the TNF-targeted biologic agents in promoting the resolution of lesions in a number of patients (see below).

These outlined findings are conclusions assembled from several different investigators, but the explanations leave significant doubts since some key points remain unexplained. In particular, the number of infiltrating inflammatory cells in the skin lesions appear to be too few to explain the widespread killing of keratinocytes. In the first place, both of the two favored pathways to cell death, viz., granzyme B- and perforin-mediated exocytosis and Fas-FasL killing, are not restricted to TEN and SJS – both pathways are upregulated in some other adverse cutaneous reactions such as maculopapular erythema where widespread cell destruction does not occur. The second inadequacy of the dual pathway explanation is the need for cell-to-cell contact for killing when there seems to be not enough inflammatory cells for this to occur. These doubts have been expressed by Chung and coworkers in Taiwan whose investigations recently provided a better understanding of the immune mechanisms and biomarkers of TEN and SJS and promise new approaches for the management of these diseases. Gene expression profiling, PCR, and immunohistochemical methods identified granulysin rather than Fas, FasL, soluble FasL, granzyme B, or perforin as the major cytotoxic molecule responsible for keratinocyte necrosis in TEN/SJS. Granulysin, a member of the saposin-like family of membrane-disrupting proteins, is a cationic cytolytic and proinflammatory protein contained in the cytolytic granules of cytotoxic T lymphocytes and NK cells. Chung and coworkers showed that granulysin from blister fluid, in the 15 kD secretory form (a precursor of the 9 kD form), was present in a concentration two to four times higher than soluble FasL, granzyme B, and perforin. Depletion of granulysin reduced cytotoxicity, and when it was injected into mouse skin, it produced TEN- and SJS-like skin necrosis. In addition to its cytotoxic effects, granulysin is a chemoattractant for other inflammatory cells and aids the expression of some chemokines and cytokines including RANTES (CCL5), MIP-1α (macrophage inflammatory protein-1α, CCL3), MCP-1 (monocyte chemotactic protein-1, CCL2), MCP-3 (monocyte chemotactic protein-3, CCL7), IL-1, IL-6, IL-10, and IFN-α.

In summary, the demonstrations of a pathogenetic mechanism for TEN/SJS and that secretory granulysin is a key toxic molecule responsible for disseminated keratinocyte killing opened the way for the development of new diagnostic and therapeutic targets for the diseases. However, important questions concerning operative mechanisms in TEN and SJS remain. For example, what are
the precise molecular mechanisms involved in the interactions between the offending drugs, HLA, and the T cell receptor? What are the precise steps between taking the drug and the secretion of granulysin? How is secretion of the cytolytic protein regulated? What are the identities of the determinants recognized in the immune processes?

In the first edition of this book after reviewing reports of the rapid resolution of skin lesions in TEN patients treated with the mAb infliximab or fusion protein etanercept, both targeted to TNF, we stated: “The beneficial effect of infliximab when used for selective TNF blockade in some cases of TEN and the importance of TNF in causing direct cytotoxicity and apoptosis must also be considered and somehow incorporated into a satisfying explanation of the pathogenesis of this intriguing toxidermia”. In a recent randomized controlled trial involving 96 patients with cytotoxic T lymphocyte (CTL)-mediated severe cutaneous adverse reactions (SCAR), including SJS and TEN, the Taiwan Severe Cutaneous Adverse Reaction (TSCAR) Consortium undertook an evaluation of the efficacy of etanercept compared to the traditional therapy, namely, corticosteroids, in CTL-mediated SCAR patients. To assess the severity and predict the mortality of patients, a severity-of-illness score (SCORTEN) was used. Etanercept decreased the predicted mortality rate from 17.7% to an observed 8.3%, and compared to corticosteroids, etanercept reduced skin healing time (14 days, etanercept; 19 days corticosteroids) and lowered the incidence of gastrointestinal hemorrhage (2.6% etanercept, 18.2% corticosteroids). Tregs expressing the FOXP3 transcription factor suppress T cell responses associated with autoimmune diseases and SJS-TEN and can suppress autoimmunity and hypersensitivity. Examinations of blister fluid and plasma showed decreases in the range 45.7–62.5% for TNF and granulysin after treatment and an increase in the CD4 + CD25hiFOX3+ Tregs population at the acute, maximum, late, and recovery stages. No significant differences were seen in the corticosteroid-treated group at these stages. Also, the Treg population at the maximum stage (8.8 days from disease onset) was significantly lower in the deceased patients than in those who survived. TNF, a proinflammatory cytokine, has a role in inducing immune responses, is highly expressed in blister fluids and plasma of SJS-TEN patients, and appears to be the upstream regulator of granulysin, the key mediator of keratinocyte detachment in the disease. It is thought that etanercept blocks the TNF pathway in granulysin expression. The authors of the study concluded that their findings provide a rationale for the clinical use of anti-TNF biologic agents in the treatment for CTL-mediated SCARs and, compared to the conventional corticosteroid treatment, etanercept is a more effective alternative therapy.

3.8.12 Vitiligo

Vitiligo (Chap. 2, Sect. 2.5.10, Fig. 2.26) may occur during the early stage of interferon therapy for hepatitis C, but it is not clear if there is a connection between the cytokine, the virus, and the destruction of epidermal melanocytes. Skin depigmentation seems to be the result of an autoimmune process together with the actions of cytokines such as interferons, IL-2, sIL-2R, IL-10, IL-13, and IL-17A and CD8+ cytotoxic T lymphocytes targeting melanocytes and expressing IFNγ. Important elements in the pathogenesis are an innate immune response induced by reactive oxygen species and the involvement of keratinocytes that release chemokines CXCL10 and CXCL16 following stimulation by IFNγ (and perhaps other cytokines) or oxidative stress. The released chemokines help to mediate the migration of CD8+ cytotoxic T cells to sites undergoing depigmentation. Activated inflammasomes such as NLRP3 in keratinocytes, which are part of the innate immune system, regulate the action of caspase 1, induce inflammation in response to a variety of danger signals including some that are host-derived, and aid the activation and migration of T cells via caspase 1-assisted secretion of IL-1β and IL-18. Serum IL-1β levels are increased in vitiligo and correlate with disease
severity. Blocking NLRP3 inflammasome activation in keratinocytes inhibited the production of cytokines by inhibiting chemotaxis of CD8+ T cells from vitiligo patients. These findings suggested that keratinocyte NLPR3 activation promotes a cutaneous T cell response that may be a target for the treatment of vitiligo.

### 3.8.13 Sweet’s Syndrome (Acute Febrile Neutrophilic Dermatosis)

The pathogenesis of Sweet’s syndrome (SS) (Chap. 2, Sect. 2.5.11, Fig. 2.27) has been difficult to elucidate, and a number of cellular and molecular mechanisms have not yet been fully defined and received general agreement. Given that the subtypes of SS are viewed as a single pathological entity under the heading of neutrophilic dermatosis, the many and various factors related to the disorder, including drugs, malignancy, and infection, suggest a multifactorial pathogenesis, but equally, a unifying mechanism such as a hypersensitivity reaction and the efficacy of corticosteroid treatments seems a possibility. However, there is little immediate and obvious immunological evidence in the form of antibodies, complement changes, and immune cytotoxic and immune complex formation for this hypothesis, but some mechanistic clues may be found in the cutaneous localization of neutrophils in the dermis and the established role of dysfunctional immune mediators in SS. Extravasation of neutrophils into the tissues proceeds via TNF-activated endothelium, interactions with selectins, intercellular cell adhesion molecules (ICAM) and integrins, and the participation of proinflammatory IL-1β. Increased levels of Th1 cytokines IFNγ, IL-1α, IL-1β, and IL-2 with decreased numbers of Th2 helper cells in the dermis are thought to be associated with neutrophil activation and localization in SS. TNF, IFNγ, IL-17, and IL-1β together produce an inflammatory response and recruit and localize neutrophils by inducing adhesion molecules and the chemoattractant CXCL8 (IL-8). As a result of TNF and IL-17 interactions and neutrophil involvement, basement membrane remodeling occurs, and matrix metalloproteinases (MMPs) are upregulated. IL-17-enhanced production of G-CSF and GM-CSF leads to further proliferation and activation of neutrophils and a host of additional proinflammatory molecules.

There is some emerging information on genetic aspects of SS, namely, neutrophilic dermatosis of dorsal hands of HLA-B54-positive Japanese patients, a link between SS and familial Mediterranean fever, and a possible association in malignancy with mutations in the isocitrate dehydrogenase 1 (IDH1) gene. SS and familial Mediterranean fever have been seen in the same patient, and mutations in the MEFV (Mediterranean fever) gene that codes for the protein pyrin which has a role in inflammation and infection lead to an inflammatory response.

### 3.8.14 Delayed Cutaneous Drug Hypersensitivity Reactions: Conclusions

In reviewing what is currently known about the pathophysiology and mechanisms underlying the T cell-mediated delayed allergic drug reactions, it is apparent that knowledge of the different cutaneous reactions is still widely incomplete and agreement, even on some basic processes, is often inconsistent or lacking. Absence of agreement on the identity of the often-bewildering number and nature of cytokines and chemokines said to be involved is particularly apparent for some of the drug-induced reactions. For the practicing clinician, especially those without specialty knowledge of immunology and dermatology, the field of drug-provoked cutaneous reactions is an area of great difficulty starting with the requirement of identifying the culprit drug, often among multiple drugs being taken. There then remains the need to undertake or order appropriate tests without further aggravating the condition; institute appropriate management measures; identify other drugs that may be
a risk; and take measures, including instruction of the patient, to avoid further reactions. A fairly recent interesting area of investigation that is particularly promising emerged from demonstrated associations between HLA alleles, certain drugs, diseases such as TEN/SJS, and different human populations. Apart from the presentation of drug or drug metabolite to T cells, HLA alleles may also be responsible for genetic susceptibilities for drug-induced cutaneous reactions. As pointed out by Chung and colleagues “Understanding the molecular mechanism of the interaction of HLA, offending drugs and TCR, as well as CTLs/NK cells activation, would facilitate the development of new approaches for the management of SJS/TEN.” With relevance to pathomechanisms and regard to classification of reactions, attention was drawn to the cell type(s) recruited during the so-called second step of drug-induced skin inflammation following the involvement of drug-specific T cells in the first step. The important involvements of eosinophils with DRESS and neutrophils with maculopapular exanthema and AGEP illustrate the point.

As discussed in Chap. 4, the demonstration or detection of individual or patterns of cytokines and chemokines is a promising approach for improving the reliability and specificity of diagnosing some drug-induced cutaneous hypersensitivity reactions. Surprisingly, this diagnostic strategy still seems to be under-utilized, but significant advancements probably depend on first reliably implicating a suitable disease-specific marker or spectrum of markers. Finally, the allergenic determinants recognized in the cellular immune processes remain largely unexplored and undefined. Identification of the structures of drug-peptide complexes presented by the MHC and fine-structural detail of drug determinants recognized by the T cell receptor remain areas in need of both investigation and secure findings. Progress on these points is needed to reliably identify potentially cross-reacting drugs for patients and offers the possibility of selecting or tailor-making interfering inhibitory or competing molecules to mitigate drug-specific reactions.

3.9 Drug-Induced Hypersensitivity and Immune Receptors

An antibody response to a chemically reactive drug or hapten is said to occur after the drug-protein complex is recognized, processed, and presented as a drug-peptide conjugate to T cells that recognize the drug-modified peptide. A low molecular weight free, unconjugated drug is thought to remain unrecognized and not equipped to elicit an immune response. For drugs, however, immunological dogma is often found wanting on at least two counts. Firstly, despite the requirement that “small” molecular weight compounds or haptons (generally less than 1000 kD) need attachment to a macromolecular carrier to become immunogenic, many haptons or drugs that remain uncomplexed and apparently too small do in fact elicit a clear immune response. Secondly, despite the pioneering findings of Landsteiner and other early immunochemists, and the conclusion that previous exposure to an allergen is a prerequisite for allergic sensitization and reactivity, the dogma of prior exposure does not always hold. Previous contact with a drug is not necessarily a prerequisite for a drug-induced immune hypersensitivity response. Although these inconsistencies were emphasized by the author and some other investigators over 30 years ago (see monograph on drug allergy, Further Reading), general acceptance of exceptions to the dogma has only recently been forthcoming.

3.9.1 Recognition and the Immune Response to Free, Unconjugated Drug

The implications for drug allergy from basic and applied research on T cell recognition of haptons commenced nearly 30 years ago were perhaps best summed up by Weltzien who, in commenting on the advances, declared simply that the work may “contribute to a better understanding of what defines an antigen as an allergy-inducing allergen.” Perhaps this will eventuate but since
the fledgling discipline of hypersensitivity research moved beyond the embryonic stage in the early half of the twentieth century and matured over a 60 year period to provide impressive insights into the effector processes in the immediate allergic response, in particular the roles of IgE antibodies, a large variety of immune cells, mast cells, basophils, inflammatory mediators, and their receptor-controlled end organ responses, the long-standing complexities of what makes an allergen an allergen has remained a subject of constant interest.

How then do small molecular weight, nonreactive chemicals such as many drugs stimulate IgE antibody production and provoke immune hypersensitivity reactions ranging from mild rashes to severe, life-threatening anaphylaxis? It must be understood that Landsteiner’s studies on the sensitizing properties of some chemicals in the form of “small” molecules linked to a protein carrier constituted the initial investigations of contact hypersensitivity and the findings and interpretations from studies on protein conjugated chemicals should not automatically be used to explain all delayed reactions and certainly not IgE antibody-mediated type I reactions. An early clue to specific immune recognition of “small,” unbound chemicals and hence drugs was the demonstration by Sinigaglia and his group of selective interaction of Ni ions with an MHC-II-bound peptide. Ni-specific T lymphocyte clones from a patient with contact dermatitis to Ni responded to the metal ions when Ni salts were presented by APC in association with DRw11.1(5) molecules. Direct evidence that Ni was bound to the MHC-associated peptide was provided by NMR spectroscopy. These results, the first direct evidence of interaction between hapten and a MHC-bound peptide, not only demonstrated a model for Ni recognition by T cells from patients with Ni hypersensitivity but also indicated that a variety of chemically reactive groups, not only reactive metal ions, might attach to MHC-bound molecules to induce MHC-restricted responses to the conjugates. Further work with Ni hypersensitivity and the occupational lung disease berylliosis established that these conditions were MHC-II-linked CD4+ delayed-type hypersensitivity responses and the high frequency of Ni-reactive T cells occurs by formation of reversible coordination complexes in which Ni interacts with the MHC and TCR via His81 of the HLA-DR α-chain and Tyr29 and Tyr94 of the CDR1α region of the TCR. This coordination complex of Ni ions directly linking the MHC peptide and TCR is similar to the action of a weak superantigen. In extending the studies on Ni to investigations on the T cell recognition of hapten, Weltzien and others have shown, somewhat surprisingly to some, that MHC-restricted hapten-specific T cell receptors react to hapten-peptide conjugates within the MHC peptide-binding groove. This opened up a new approach for studying the molecular mechanisms underlying hapten recognition by T cells.

3.9.2 Abacavir and the MHC-Presented Altered Peptide Model of Drug Hypersensitivity

More recently, some interesting HLA associations in drug hypersensitivities have been reported. A strong association of hypersensitivity to the guanosine-related prodrug and reverse transcriptase inhibitor abacavir was found with the well-defined 57.1 MHC haplotype encoding the MHC class I allotype HLA-B*57:01 (Chap. 1, Sect. 1.5). Multi-organ reactions to abacavir, termed abacavir hypersensitivity syndrome or AHS, manifests as fever, rash, malaise, nausea, and diarrhea. It occurs in approximately 2–8% of patients with human immunodeficiency virus-l (HIV-1) infection and can be severe enough to cause death in some rechallenged patients. Abacavir-specific CD8+ T cells secrete TNF and IFNγ and are cytotoxic to abacavir-APCs. In a 2008 study, implication of the fine-structural specificity of the 6-cyclopropylamino group of abacavir as a possible reactive site in the HLA restricted CD8+ T cell response was demonstrated by lack of recognition of the abacavir structural analogs carbovir, didanosine, and gua...
nosine by abacavir-reactive T cells (Fig. 3.27). Specificity of the interaction was further mapped to the F pocket (one of six, termed A–F), of the MHC-1 antigen-binding cleft where it was thought that abacavir, or a metabolite, binds to one or more self-peptides. At that stage, whether the binding was covalent or not had yet to be determined. It was predicted that the demonstration that AHS is an MHC-I-restricted cellular hypersensitivity response mediated by CD8+ T cells might prove to be a forerunner for our better understanding of the basis of immune receptor recognition in drug hypersensitivities and, more specifically, for elucidating the pathogenesis of some of the life-threatening drug-induced systemic reactions such as TEN and SJS.

Results explaining the molecular basis of AHS may have implications for understanding the origins and general molecular processes of autoimmunity. AHS is mediated by abacavir-specific activation of cytotoxic CD8+ T cells that require HLA-B*57:01 antigen-presenting cells, but abacavir-specific T cells are not activated by cells expressing the closely related allotypes HLA-
B*57:02, HLA-B*57:03, and HLA-B*58:01 each of which is insensitive to abacavir and not linked to AHS. Two amino acid residues, Asp114 and Ser116, distinguish HLA-B*57:01 from the abacavir-insensitive alleles, and abacavir reacts with these two amino acid residues. In extending the finding that the abacavir-HLA-B*57:01 association results from specific binding of the drug to the HLA F pocket, amino acid sequences of a large number of HLA-B*57:01- and HLA-B*57:03-bound peptides from untreated and abacavir-treated cell lines were determined. Abacavir-treated HLA-B*57:01 cells, but not treated HLA-B*57:03 cells, contained unmodified drug but no metabolites indicating that abacavir bound non-covalently and specifically with HLA-B*57:01. Up to 25% of the peptides bound to HLA-B*57:01 following treatment with abacavir proved to be different to those before treatment, but a change was not seen with peptides bound to HLA-B*57:03 or HLA-B*58:03. These results again suggested that abacavir binds specifically to the antigen-binding cleft of HLA-B*57:01, and this alters the repertoire of self-peptides bound by HLA-B*57:01 but not the repertoire bound by the other HLA alleles. Sequences of peptides that bind HLA-B*57:01 contained Trp→Phe at the C-terminus (PΩ), but for HLA-B*57:03 PΩ was reversed, i.e., Phe→Trp. After abacavir treatment, an increased number of peptides with Ile or Leu at PΩ bound HLA-B*57:01. In another recent study, peptides eluted from an HLA-B*57:01 single allele-transfected cell line treated or not treated with abacavir were analyzed. A significant number of peptides with Val at the C-terminus were identified in the presence of abacavir, but no peptides with Val at the C-terminus were found in untreated cells. Significant numbers of peptides with terminal Ile and fewer peptides with Trp and Phe also occurred in the presence of abacavir. Taken together, the results of the abacavir-HLA binding studies indicate that the drug positions itself at the bottom of the antigen-binding cleft extending, via the cyclopropyl moiety (Fig. 3.27), into the F pocket and changing the shape of the cleft. This results in preferred binding of smaller amino acids, an alteration in the repertoire of self-peptides that bind HLA-B*57:01 and a T cell response to self-proteins presented only in the presence of abacavir. Extension of this investigative approach to the anti-epileptic carbamazepine, a drug strongly associated with HLA-B*15:02 (see below), showed that the drug binds to this allotype and, again, an altered repertoire of presented self-peptides results. This raises the possibility that antigen-presenting molecules may be susceptible to modulation by drugs (and perhaps even toxins, environmental chemicals, etc.) causing altered T cell immunity. If this is a general mechanism, investigations of associations of other drug hypersensitivities with antigen-presenting molecules may reveal further fascinating insights into some poorly understood, unpredictable, and potentially life-threatening adverse drug reactions and ultimately lead to a better understanding of the immunopathogenesis of autoimmunity, infectious diseases, and cancer.

3.9.3 Carbamazepine and Other HLA-Drug Hypersensitivity Associations

Table 3.3 sets out a list of some known HLA-associated drug hypersensitivities. In addition to the associations of HLA-B*57:01 with abacavir hypersensitivity and flucloxacillin-induced liver injury (Chap. 5, Sect. 5.1.10), HLA-DRB1*15:01 with amoxicillin-clavulanic acid, lumiracoxib-induced hepatotoxicity, and HLA-B*58:01 with allopurinol-induced SJS (see below), HLA-B*15:02 is strongly associated with carbamazepine-induced SJS and TEN. The generality of the abacavir-HLA binding results was tested in a preliminary way in an examination of the well-established strong association between HLA-B*15:02 and carbamazepine-induced SJS/TEN in Asian populations. A non-covalent association between carbamazepine and HLA-B*15:02 was established by purifying HLA-B*15:02-peptide complexes and sequencing the bound peptides. This revealed a preference for smaller amino acids at key positions and significant increases in
| Causative drug            | Hypersensitivity reaction | HLA alleles                                      | Ethnicity                                      |
|--------------------------|--------------------------|-------------------------------------------------|-----------------------------------------------|
| Abacavir                 | Abacavir hypersensitivity syndrome | HLA-B*57:01                                     | European descent, African-American, Hispanic descent |
| Allopurinol              | SCAR                     | HLA-A*33:03, HLA-B*58:01, HLA-C*03:02, HLA-B*58:01 | European descent, Han-Chinese                 |
| Amoxicillin-clavulanic acid | DILI                  | HLA-A*02:01, HLA-A*30:02, HLA-DQA1*01:02, HLA-DQB1*06:02, HLA-DRB1*15:01, HLA-DRB5*01:01 | Japanese, Thai                                |
| Carbamazepine            | SJS/TEN                  | HLA-A*31:01, HLA-A*31:01, HLA-B*07:02            | European descent                             |
|                          | MPR                      | HLA-A*02:01, HLA-A*24:02, HLA-A*33:03, HLA-B*15:02, HLA-B*15:11, HLA-B*40:01, HLA-B*58:01, HLA-C*01:02, HLA-C*03:02, HLA-C*08:01, HLA-DQA1*03:03, HLA-DQB1*04:05, HLA-DQB1*07:01, HLA-DQB1*12:02, HLA-A*31:01, HLA-B*51:01 | Han-Chinese                                  |
|                          | SJS/TEN, MPR, EEM        | HLA-A*31:01, HLA-B*15:02                         | Indian                                        |
|                          | SJS/TEN, SCAR            | HLA-A*02:06, HLA-A*31:01, HLA-B*51:01, HLA-B*15:11, HLA-B*39:02 | Japanese                                      |
|                          | SCAR                     | HLA-A*31:01, HLA-B*15:11                         | Korean                                        |
|                          | SJS/TEN, SJS/TEN, DRESS  | HLA-B*15:02, HLA-B*46:01                         | Malay, Thai, Vietnamese                      |
|                          |                         | HLA-B*38:01/02/11                               | Thai                                          |
| Sulfamethoxazole         | SJS/TEN                  | HLA-B*15:02                                     | European descent                             |
|                          | SJS/TEN                  | HLA-B*15:02, HLA-C*06:02, HLA-C*08:01           | Thai                                          |

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SCAR severe cutaneous adverse reactions (AGEP, DRESS and SJS/TEN); DILI drug-induced liver injury; SJS/TEN Stevenson-Johnson syndrome/toxic epidermal necrolysis; DRESS drug reaction with eosinophilia and systemic symptoms; MPR maculopapular rash
the presence of some hydrophobic residues. Comparisons with HLA-B*15:01 show that this allele is not associated with carbamazepine-induced SJS, and an important difference between HLA-B*15:02 and HLA-B*15:01 is at position 156 (Leu for the former, Trp for the latter) near where the drug is thought to bind in HLA-B*15:02.

The carbamazepine-HLA-B*15:02 interaction has also been used by S-I Hung and collaborators in Taiwan as a model for the study of the pathologic role of HLA in delayed-type drug hypersensitivity. No intracellular metabolism or antigen processing was detected in the interaction between carbamazepine and HLA-B*15:02 in patients with the bullous skin conditions, and surface plasmon resonance assays showed that HLA-B*15:02, but not other HLA-B recombinant proteins, directly binds carbamazepine and the structurally related carbamazepine 10,11-epoxide. For drug presentation and activation of cytotoxic T lymphocytes, endogenous peptides in the antigen-binding groove were shown to be necessary. This is in contrast to abacavir which binds to HLA-B*57:01 without peptide loading. Modeling suggested that the Arg62 side chain, located in the B pocket of the HLA-B*15:02 protein, was the most likely binding site for carbamazepine by forming a hydrogen bond with the ketone of its 5-carboxamide group on the tricyclic ring. Specific recognition of this group was supported by results obtained with selected structural analogs.

Allopurinol is an important treatment for hyperuricemia-related diseases, being used to lower uric acid in gout, kidney stones, and Lesch-Nyhan syndrome. Unfortunately, the drug is also a frequent cause of adverse drug reactions, accounting, it is said, for up to 5% of severe cutaneous adverse reactions. Reactions include drug hypersensitivity syndrome, SJS, and TEN. In a Taiwanese study designed to identify genetic markers for allopurinol cutaneous reactions, the HLA-B*58:01 allele was identified in 100% of 51 patients with allopurinol-induced serious reactions but in only 15% (20) of 135 tolerant patients. These results indicate that in Han Chinese, allopurinol is strongly associated with HLA-B*58:01 and this allele is an important genetic risk factor for the serious cutaneous reactions with systemic symptoms (see below).

The NSAID and phenylbutazone derivative, feprazone, was found to be associated with HLA-B22 in a Scandinavian study – 93% of patients with a fixed drug eruption caused by the drug were HLA-B22 positive, but no patients with fixed drug eruptions to other drugs were positive to HLA-B22, and this allele was found in 4% of healthy controls. However, a number of factors, including the absence of HLA-B22 in 7% of the Scandinavian patients with feprazone-induced fixed drug eruption, need further scrutiny before feprazone-HLA-B22 can be taken as a diagnostic marker. At the least, more extensive population studies are needed.

Other drug hypersensitivities or intolerances thought or claimed to be associated with HLA class I and/or class II alleles include sulfamethoxazole with SJS/TEN for patients of European and Thai descent (Table 3.3), trimethoprim-sulfamethoxazole with HLA-A30, aspirin with a number of different haplotypes (see Chap. 9, Sect. 9.7), hydralazine-induced systemic lupus erythematosus with HLA-DRw4, and nevirapine hypersensitivities (including hepatitis and DRESS) with a surprising and confusing array of associations.

It has been claimed that two mechanisms for T cell stimulation are involved in the association of flucloxacillin with HLA-B*57:01 in drug-induced liver injury, a hapten mechanism and a direct interaction of drug with immune receptors (Chap. 5, Sect. 5.1.10). The former mechanism is said to involve stable (covalent) presentation of flucloxacillin on HLA molecules, resistance to washing, and dependence on proteasomal processing. Hapten-like immune responses were obtained in 35% of HLA-B*57:01 flucloxacillin T cell lines. The second mechanism, a non-hapten one, is restricted to HLA-B*57:01 and characterized by non-covalent presentation of drug, direct and reversible interaction with the TCR or HLA, independence of proteasomal processing, and
immediate activation of T cell clones by flucloxacillin.

Support for MHC class I as a major activation pathway via a route independent of antigen processing has been forthcoming from a study of allopurinol-induced severe cutaneous reactions associated with HLA-B*58:01. Allopurinol-generated T cell lines reactive with both allopurinol and its metabolite oxypurinol were employed. Fixing with paraformaldehyde did not affect oxypurinol-specific T cell proliferation, suggesting antigen processing was not involved in the interaction between oxypurinol and HLA-B*58:01. Site-directed mutagenesis indicated that Arg97 located between the E and C pocket of HLA-B*58:01 is important for drug binding.

This brief summary of findings so far of the role of HLA in severe cutaneous drug reactions highlights the need for extensive phenotyping studies and investigations in much larger populations. As already demonstrated, results can lead to the identification of high-risk populations and patients and provide insights into the immunopathogenesis of some severe cutaneous and systemic drug reactions and syndromes.

### 3.9.4 The Question of Direct Drug Activation of T Cells Without Involvement of a Specific Peptide

Drawing on earlier speculations on the absence of prior sensitization in many drug reactions and the seminal studies of MHC-restricted metal ion and drug hypersensitivities mediated by T cell activation, others have suggested some modifications to the possible cellular and drug interactions involved in drug-specific recognition by cells of the immune system. A number of observations including the prevention of T cell activation after removal of drug by washing, rapid calcium influx into T cells after exposure to drug, and the fact that glutaraldehyde-fixed APC can still present drug have led to the suggestion that T cells rather than APCs recognize free, unprocessed parent drug in allergic individuals. Proponents of the so-called p-i concept (derived from the proposed direct pharmacological interaction of drugs with immune receptors) state that “drugs bind specifically and reversibly to some of the highly variable antigen-specific TCR in a direct way, instead of covalently modifying the MHC-peptide complex.” Direct and in-depth experimental findings of the sort presented in the Ni, abacavir, and carbamazepine/HLA-B*15:02 investigations to support this hypothesis are lacking, and with, for example, abacavir bound at the bottom of the HLA binding groove, it is difficult to see how the drug can directly contact, or by itself directly influence, the T cell receptor. Recently the proposed model restricted to T cell binding appears to have been modified to acknowledge, and accommodate, the association and presentation of some drugs, or drugs with peptide, in the MHC peptide-binding groove. The revised definition now refers to a drug interacting directly and reversibly via non-covalent bonds with the TCR or HLA.

### 3.10 Desensitization of Drug-Allergic Patients: Immediate and Delayed Reactions

An adverse reaction to a drug can be a major problem in efforts to achieve successful treatment for common and important diseases including infections, arthritides, allergies, and malignancies. Adverse drug reactions occur frequently, and as the number, chemical nature, and novel pharmacological actions of registered drugs continue to increase, such reactions can seriously interrupt therapy and leave patients with less than optimal treatments. If no alternative effective drug is available, rapid drug desensitization (RDD) can provide an effective and safe means to continue vital therapies. The aim of desensitization is to administer increasing amounts of drug in an incremental and stepwise manner while at the same time avoiding or minimizing life-threatening or even lesser adverse symptoms. When successful, the procedure induces temporary tolerance to the drug allowing treatment to continue with optimal dosages.
Although desensitization of drug-induced hypersensitivity is contraindicated in type II and type III reactions, it is used quite extensively in patients with type I hypersensitivities where there is some understanding of underlying mechanisms and much less often in type IV reactions where mechanistic detail is often severely lacking.

In considering possible mechanisms leading to rapid desensitization to a drug in allergic type I immediate drug reactions, the mast cell and basophil appear to be the cells most likely to be involved. IgE antibody levels do not decrease, and a simple reduction in drug-reactive IgE cannot explain desensitization. RDD appears to result in the mast cells becoming temporarily tolerant to the drug. Although some important results pertaining to signaling were obtained 10–15 years ago (see below), a comprehensive explanation of how RDD tolerizes mast cells/basophils and interferes with their activation is lacking mechanistic detail. One investigative approach involved the delivery of increasing quantities of antigen at fixed time intervals to mouse bone marrow mast cells in vitro together with the monitoring of granule release by detection of β-hexosaminidase and the metabolism of prostaglandins and leukotrienes. Both of these indicators were inhibited by desensitization, and this was achieved by incremental increases in dosage. Importantly, the presence of antigen was necessary for desensitization – as long as antigen was maintained, desensitization was maintained. Mast cells desensitized to dinitrophenol did not release preformed and de novo synthesized mediators such as TNF and IL-6. This may help to explain why desensitized patients are not at risk of a delayed reaction. Experiments in which mast cells were sensitized to dinitrophenol and ovalbumin showed that ovalbumin-desensitized cells responded fully to dinitrophenol, proving antigen specificity and that signaling transduction pathways have not been impaired during desensitization. Furthermore, FcεRI-bound antigen-specific IgE molecules did not disappear from the cell surface during desensitization after becoming bound to small doses of antigen. These results are reassuring in that they support both the proposed inhibition of the mast cell response and the basis for the RDD procedures currently used. Over many years, a number of other mechanisms have been proposed to explain the state of clinical tolerance resulting from the practice of desensitization. The list includes the formation of IgG blocking antibodies, consumption or blocking of the drug-reactive IgE antibodies by the gradually increasing quantities of administered drug, tachyphylaxis or depletion of the released mediators, hapten inhibition by monovalent penicillin-protein conjugates, and desensitization of mast cells and basophils by gradually increasing quantities of multivalent drug-carrier complex.

In 1990 in a study of releasing and nonreleasing human basophils, MacGlashan and colleagues reported that nonreleasing basophils lack an early component of the signal transduction pathway stimulated by cross-linking of IgE. Kepley and colleagues then found that basophils from the nonreleaser phenotype lacked detectable Syk (Sects. 3.2.2 and 3.4.2), a protein tyrosine kinase involved in the initiation of FcεRI-mediated secretion. Further studies with human skin mast cells showed that FcεRI became nonresponsive with antigen concentrations that did not induce release and this nonresponsive state, as with basophils, correlated with reduced Syk levels. Also, reversal of IgE unresponsiveness in mast cells to FcεRI-mediated responsiveness was shown to parallel increased Syk levels. In a 2005 paper, Kepley concluded: “This mechanism may explain why low concentrations of allergen given to patients during allergen immunotherapy induce FcεRI nonresponsiveness and therapeutic benefit without inducing systemic anaphylaxis.” Subsequently, in studying subthreshold desensitization of human basophils, MacGlashan also concluded that two critical signaling molecules, Syk and FcεRI, were lost and that whereas loss of Syk is not influenced by IL-3, receptor internalization is induced by the cytokine.

Note that desensitization to a drug does not result in long-term tolerance to its adverse effects. This means that patients need the desensitization procedure to be repeated each time they are again exposed to the drug. However, if the medication is maintained, for example, by daily dosage with
pharmacologically active levels, the desensitization state can be maintained.

Protocols for desensitization to different groups of drugs are shown in this book for β-lactams in Chap. 5 and for some important antimicrobials (Chap. 6), NSAIDs (Chap. 9), contrast media (Chap 10), proton pump inhibitors (Chap. 12), small molecule chemotherapeutic agents (Chap. 15), and a range of biologics including monoclonal antibodies, cytokines, fusion proteins, and the hormone insulin (Chaps. 13 and 14).

Desensitization of cell-mediated cutaneous reactions is even less well understood than desensitization of immediate allergic reactions, and with considerable doubts about its efficacy and safety, it is not surprising that the procedure is relatively little used. Currently, the procedure tends to be restricted to uncomplicated exanthems without systemic symptoms and fixed drug eruptions, but (in addition to trimethoprim-sulfamethoxazole (TMP-SMX) and antibiotics [see below]) some drugs used to treat epilepsy, gout, rheumatoid arthritis, Crohn’s disease, and ulcerative colitis have been successfully employed to desensitize patients. It is generally accepted that culprit drugs involved in the severe and life-threatening delayed hypersensitivity reactions SJS and TEN but also DRESS, and perhaps even AGEP, are contraindicated for desensitization. Other contraindicated conditions include underlying autoimmune disorders; pre-existing severe renal or hepatic impairment; severe cardiac disease/hemodynamically unstable patients; patients with extensive mucosal ulcersations, cutaneous or systemic vasculitis, drug fever, or generalized lymphadenopathy; and patients with drug-induced internal organ involvement such as hepatitis, nephritis, pneumonitis, cytopenias (agranulocytosis, thrombocytopenia, anemia), or severe eosinophilia.

While it is said that slow desensitization protocols are more effective, it is likely that rush protocols have not been attempted with many drugs. As a general rule, it is wise to avoid the offending drug, but in a few cases, there is little or no choice if there is no suitable alternative and the drug(s) in question has proven to be a very effective treatment and safe. Examples of this situation are seen with the use of TMP-SMX as a prophylactic agent for Pneumocystis carinii in HIV patients and some antibiotic treatments for cystic fibrosis such as piperacillin with tazobactam, meropenem, aztreonam, ceftazidime, and tobramycin. The protocol shown in Table 3.4 has proved to be a successful desensitization procedure for a significant proportion of HIV patients who experienced only mild-moderate adverse reactions that did not limit treatment. A 6 h oral graded challenge beginning at least 1 month after TMP-SMX hypersensitivity reactions in 44 HIV-infected patients without premedication has also been claimed to be safe and effective with a high success rate of 95% (Table 3.5) (see also Chap. 6, Sect. 6.3.1.3).

Table 3.4 Trimethoprim-sulfamethoxazole (TMP-SMX) dose-escalation regimen in human immunodeficiency virus-infected patients

| Dosing level | Portion of single-strength TMP-SMX (%) | Amount (frequency) of pediatric suspension (ml) | Total TMP dose (mg) | Total SMX dose (mg) |
|--------------|----------------------------------------|-----------------------------------------------|---------------------|---------------------|
| 1            | 12.5                                   | 1.25 (q.i.d.)                                 | 10                  | 50                  |
| 2            | 25                                     | 1.25 (b.i.d)                                  | 20                  | 100                 |
| 3            | 37.5                                   | 1.25 (t.i.d)                                  | 30                  | 150                 |
| 4            | 50                                     | 2.5 (b.i.d)                                   | 40                  | 200                 |
| 5            | 75                                     | 2.5 (t.i.d)                                   | 60                  | 300                 |
| 6            | 100                                    | 1 single-strength tablet                      | 80                  | 400                 |

Adapted from Leoung GS, Stanford JF, Giordano MF, et al. Trimethoprim-sulfamethoxazole (TMP-SMZ) dose escalation versus direct challenge for Pneumocystis Carinii pneumonia prophylaxis in human immunodeficiency virus-infected patients with previous adverse reaction to TMP-SMZ. J Infect Dis. 2001;184:992–7, with permission from Oxford University Press

Note: Each dosing level is a daily dose. For successful completion of the reintroduction phase, patients must have taken each dose level at least once. Patients were permitted to repeat dose levels once; dose levels were completed in increasing increments, and the level 6 dose was taken no later than day 13 of the reintroduction phase. Patients were permitted to withhold study drug for 2 days during the reintroduction phase (withholding study drug for >2 days during reintroduction resulted in permanent discontinuation). Patients were required to take an antihistamine during dose escalation. b.i.d., twice daily; t.i.d. 3 times daily

aRelative to 1 single-strength tablet (TMP 80 mg, SMX 400 mg)
bSuspension containing TMP 40 mg and SMX 200 mg/ml
cTablet containing TMP 80 mg, SMX 400 mg
Desensitization was recently attempted in 20 patients who exhibited cutaneous reactions to the anticonvulsant oxcarbazepine and who participated after HLA genotyping to detect predisposition to SJS. Commencing with a dose of 0.1 mg per day, dosage was increased incrementally until days 73–82 when a maximum of 612 mg of oxcarbazepine was administered. Nineteen patients completed the course of desensitization without severe allergic reactions. Patients were followed for 1–6.7 years (4.6 ± 1.2 years): at 1 year seizures had reduced by more than 50% in 16 patients; at 3 years, 14 patients had a reduction in seizure frequency of ≥50%, and 8 patients remained seizure-free after 3 years.

Successful desensitizations to fixed drug eruptions (FDEs) have been achieved. In one case involving multiple lesions caused by allopurinol, the patient was given 10 μg of allopurinol twice a day for 2 days and then increased every second day until, at 25 mg, an erythematous reaction appeared. This was managed by decreasing the dose to 10 mg before increasing it every 7 days to 25 mg, then 50 mg, and finally 100 mg. During desensitization the patient was given 10 mg of prednisone daily. The desensitization was successfully completed in about 6 weeks. After desensitization the patient was able to tolerate allopurinol 200 mg daily without recurrence of FDE. As with most other cases of FDE, T cells from the pigmented lesions before desensitization were mainly CD8+ cells, but in this case, CD4+ cells, about half expressing CD25, were seen after desensitization. A subset of CD25 + CD4+ T cells have a role in regulation of the immune response suggesting that their presence in the FDE lesions may have been related to the induction of desensitization. In another successful desensitization protocol for allopurinol, the patient was started on a dose of prednisone, 10 mg/day, and allopurinol 50 μg/day for 3 days. This was increased to 100 μg and, then at 3-day intervals, to 200 μg, 500 μg, 1 mg, 5 mg, 10 mg, 25 mg, 50 mg, and 100 mg. The patient continued to receive prednisone during the desensitization period and during a 1-month allopurinol 100 mg/day maintenance period.

For more information on drug-induced type II hypersensitivities, see Chaps. 2, Sect. 2.3, and Chap. 15, Sects. 15.2.2 and 15.3.2.

The classical drug-induced type II hypersensitivity is the well-known immune cytotoxic reaction to high doses of penicillin that results from binding of the drug to red cells. This causes the red cells to be recognized as foreign, resulting in IgM and IgG antibodies reacting with the drug-cell membrane protein complex. The antibody-antigen complexes so formed activate the classical complement pathway causing cell lysis and death, and the antibody-coated red cells can interact with macrophages leading to Fc-mediated cell destruction by the reticuloendothelial system.

### 3.11.1 Drug-Induced Immune Hemolytic Anemia

Another example of a type II cytotoxic antibody-mediated drug reaction when the drug appears to form an antigenic complex with the red cell surface is drug-induced immune hemolytic anemia (DIIHA). The drugs most frequently associated with DIIHA are some cephalosporins (especially cefotetan and ceftriaxone) and penicillins (especially piperacillin). DIIHA can also be associ-

| Table 3.5 One-day trimethoprim (TMP)-sulfamethoxazole (SMX)-oral graded challenge | Dosea (μg) | Dosea (mg) |
|---|---|---|
| 1 | 1/0.2 | 7 | 1/0.2 |
| 2 | 3/0.6 | 8 | 3/0.6 |
| 3 | 9/1.8 | 9 | 9/1.8 |
| 4 | 30/6.0 | 10 | 30/6.0 |
| 5 | 90/18 | 11 | 90/18 |
| 6 | 300/60 | 12 | 300/60 |

Adapted from Demoly P, Messaad D, Sahla H, et al. Six-hour trimethoprim-sulfamethoxazole-graded challenge in HIV-infected patients. J Allergy Clin Immunol. 1998;102:1033–6, with permission from Elsevier.

*Dosages given at half-hourly intervals

Pediatric solution containing TMP 8 mg and SMX 40 mg/ml

From day 2, patients were given TMP-SMX 80/400 mg once daily
ated with red cell autoantibodies induced by the drug affecting the immune system without becoming bound to the red cell surface, that is, the drug does not participate in the antigen-antibody reaction. Such antibodies are referred to as drug independent. Prototype drugs involved in drug-independent autoantibody formation are methyldopa and the chemotherapy drug, fludarabine. In this form of DIIHA, the clinical and laboratory findings are identical to autoimmune hemolytic anemia. It is not known why drugs sometimes induce non-independent autoantibodies to red cells or what mechanism is involved. The mechanism of DIIHA when the drug participates as the antigen is thought to proceed by attachment of the drug to the red cell in vivo, interaction with drug-reactive antibodies (usually IgG but may be IgM), and subsequent Fc-mediated cell destruction and clearance by macrophages. Activation of complement may occur leading to intravascular lysis and renal failure. Understanding the mechanisms involved in the action of drug-dependent antibodies is complicated by the observation that drugs that cause some of the worst reactions, including hemolysis, renal failure, disseminated intravascular coagulation, and death, appear to proceed via a different mechanism, often involving complement-activating antibodies. The so-called “unifying hypothesis” has been advanced to explain all three types of antibodies implicated in DIIHA. This hypothesis is based on known findings dating back to Landsteiner of the generation of three populations of antibodies to an injected hapten-protein complex – one population to the hapten, one to hapten plus carrier determinants, and one to the protein carrier. A further, and more recent, proposed mechanism for DIIHA is based on non-immune adsorption of the drug onto the red cell membrane. Cefotetan, often used prophylactically in some surgical procedures, is the most common cause of DIIHA with another cephalosporin, ceftriaxone, the second most common cause. For antibody binding to occur, the presence of the drug is essential – in the absence of the drug, antibodies do not bind to the platelet surface, and thrombocytopenia does not occur. It remains uncertain whether the antibodies are directed to the drug alone or to the complex of drug and platelet glycoprotein. A third mechanism of drug-induced thrombocytopenia is seen with the anti-platelet GPIIb/IIIa inhibitor drugs lotrafiban, tirofiban, and eptifibatide, the novel cyclic heptapeptide from the venom of the southeastern pygmy rattlesnake. By binding to the glycoprotein complex, these drugs induce a conformational change and a new determinant to which antibodies bind and cause cell destruction. The drug does not

3.11.2 Drug-Induced Thrombocytopenia

In addition to erythrocytes, other cells including platelets (thrombocytes) and some hematopoietic precursor cells can be affected by drug-induced type II hypersensitivity reactions. Drug-induced thrombocytopenia, for example, is increasing as more drugs are released and used. A number of different mechanisms appear to be involved. Drugs may bind covalently to the platelet membrane producing a hapten-glycoprotein conjugate with an antigenic determinant(s) that is recognized by antibody. Drugs implicated in this form of thrombocytopenia include penicillins and cephalosporins in particular. Quinine, quinidine, sulfonamides, and NSAIDs may interact non-covalently with platelet membrane glycoproteins, including the von Willebrand factor receptor GPIb-IX-V (GP for glycoprotein) and activated integrins GPIIb/IIIa, forming drug-glycoprotein non-covalently linked complexes. For antibody binding to occur, the presence of the drug is essential – in the absence of the drug, antibodies do not bind to the platelet surface, and thrombocytopenia does not occur. It remains uncertain whether the antibodies are directed to the drug alone or to the complex of drug and platelet glycoprotein. A third mechanism of drug-induced thrombocytopenia is seen with the anti-platelet GPIIb/IIIa inhibitor drugs lotrafiban, tirofiban, and eptifibatide, the novel cyclic heptapeptide from the venom of the southeastern pygmy rattlesnake. By binding to the glycoprotein complex, these drugs induce a conformational change and a new determinant to which antibodies bind and cause cell destruction. The drug does not
physically form part of the determinant. Another inhibitor of platelet activation sometimes administered is abciximab, the Fab fragment of a chimeric human-mouse monoclonal antibody that binds to the platelet glycoprotein receptor GPIIb/IIIa. Some patients, even without prior exposure to the monoclonal agent, react to the mouse component of the hybrid, supporting the belief that natural antibodies may be involved in the recognition. Such recognition of murine antigens on a chimeric human-mouse antibody fragment is similar to the recognition by natural antibodies of the chimeric monoclonal antibody cetuximab. This humoral form of immune-mediated drug-induced thrombocytopenia is regarded as drug-specific since the antibodies are formed against the drug itself and platelets are destroyed in the process. In a fifth mechanism, the drug induces the formation of autoantibodies to glycoproteins on the platelet surface. The antibodies bind to the platelet antigens without participation of the drug, and the resultant thrombocytopenia can persist when the drug is withdrawn. The prototype drugs in this category are gold and procainamide. Finally, heparin and heparin-like drugs can induce thrombosis by binding to surface-bound soluble platelet factor 4 (PF4), a small chemokine CXCL4 that promotes coagulation and is released from the alpha granules of activated platelets during platelet aggregation. Antibodies to the heparin-PF4 complex bind to receptors on the platelet surface via their Fc pieces producing platelet activation. This mechanism is basically different from the other five described mechanisms in that activation and aggregation of platelets are the result rather than cell lysis and hemorrhage making the reaction more like a type III than a type II hypersensitivity response.

### 3.11.3 Agranulocytosis

Acute agranulocytosis is rare, but when it does occur, drugs are responsible in more than 70% of cases. In its immune form, antibodies are produced to circulating neutrophils and/or myeloid precursor cells. Immune-mediated agranulocytosis is rapid in onset with symptoms generally occurring within a few days. Drugs commonly associated with the condition include quinine, quinidine, β-lactams, pyrazolones, propylthiouracil, clozapine, ticlopidine, carbamazepine, chlorpromazine, and some sulfonamides. Numerous other drugs have been implicated in one or only a few cases. Several mechanisms have been advanced although detailed and convincing evidence is not always offered. Some of the implicated drugs such as penicillins and aminopyrine are thought to act as haptens that elicit antibody formation against neutrophils and their subsequent destruction. In the case of aminopyrine-induced agranulocytosis, antibodies are directed to neutrophil cell membrane antigens modified by a reactive metabolite of the drug. Antibody recognition of metabolites was also demonstrated for metamizole and diclofenac in cases of agranulocytosis induced by these drugs. In addition to drug-dependent antibodies of the IgG and/or IgM class, autoantibodies were found in 13 cases of drug-related agranulocytosis due to penicillins, dimethylaminophenazone, propyphenazone, metamizole, and diclofenac. In the case of clozapine-induced agranulocytosis, the drug is converted to the reactive nitrenium ion which binds to cellular proteins and accelerates apoptosis of neutrophils. Propylthiouracil was shown to lyse neutrophils via a complement-dependent mechanism. An immune mechanism does not seem to be involved with drugs such as ticlopidine, busulfan, chlorpromazine, and methimazole each of which has a direct toxic effect on myeloid precursors.

### 3.12 Type III Hypersensitivity Drug Reactions

For more information on drug-induced type III hypersensitivities, see Chap. 2, Sect. 2.4, and Chap. 15, Sects. 15.2.3 and 15.3.2.

#### 3.12.1 Serum Sickness

Serum sickness can occur in response to foreign proteins such as streptokinase and to antitoxins, antivenins, and vaccines. Antigen-antibody
complex-mediated reactions occur in some cases that closely resemble classical serum sickness. Penicillin has long been known to become antigenic by conjugating to proteins in vivo to produce drug-protein complexes that mediate type III hypersensitivity reactions. Thus, it can be said that penicillins can cause all four types of hypersensitivity reactions. Other drugs that produce similar serum sickness-like reactions include cephalosporins, sulfonamides, ciprofloxacin, tetracycline, lincomycin, NSAIDs, carbamazepine, allopurinol, thiouracil, propranolol, griseofulvin, metronidazole, furoxone, captopril, gold salts, methyldopa, halothane, fluoxetine, barbiturates, and monoclonal antibodies. β-Lactam drugs are considered the most common cause of serum sickness elicited by non-proteins, but drugs by themselves are thought to be poor antigens for the production of the good antibody responses necessary to induce serum sickness. Circulating antigen-antibody complexes are formed after drugs become protein-bound in vivo and stimulate IgG and/or IgM antibodies. The liberation of vasoactive amines is thought to play a part in tissue deposition. Antigen also interacts with complementary IgE antibodies on mast cells and basophils leading to the release of PAF and other mediators, platelet aggregation, and further release of histamine and serotonin. The resulting increase in vascular permeability facilitates the deposition of immune complexes which, in turn, produces complement activation, the formation of C3a and C5a, an influx of inflammatory cells to the sites of immune complex deposition, and release of further inflammatory mediators. Drug immune complexes are normally rapidly cleared via the antibody Fc piece or complement binding to cells of the reticuloendothelial cells, but if this does not occur, for example, because of the high concentrations of immune complexes, deposition of complexes in glomeruli, arteries, endocardium, spleen, and other organs and influx of inflammatory cells may result. In a graph that relates the time of occurrence of tissue lesions to the clearance of antigen and developing antibody production, Fig. 3.28 summarizes the immunologic events in the patient after antigen exposure. Serum concentration of protein-bound drug initially decreases sharply as a result of intravascular and extravascular equilibration, and levels continue to decrease normally as the protein is catabolized until antibody levels increase, causing rapid immune elimination. The dashed red line in Fig. 3.28 at about day 6 represents the course of antigen decline in the absence of antibody-mediated antigen elimination. From about day 14, soluble circulating complexes of antigen with IgG or IgM form and may begin to

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**Fig. 3.28** Relationship between antigen introduction, subsequent immunological events, and time in serum sickness

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| Days | Antigen or antibody in serum (%) |
|------|---------------------------------|
| 2    | 100                             |
| 4    | 10                             |
| 6    | 1                              |
| 8    | 0.1                            |

| Days | Antigen-Antibody complexes & serum sickness reaction |
|------|-----------------------------------------------------|
| 2    | 100                                                 |
| 4    | 10                                                  |
| 6    | 1                                                   |
| 8    | 0.1                                                 |
deposit in a number of tissue sites leading to the clinical manifestations and pathologic findings of serum sickness.

### 3.12.2 Drug-Induced Vasculitis

Drug-induced vasculitis (DIV) is another manifestation of a type III response. An extensive list of drugs from almost every drug group has been implicated including an increasing number of recombinant biologics. A proportion of small-vessel vasculitis patients have drug-induced anti-neutrophil cytoplasmic antibodies (ANCA) (Chap. 2, Sect. 2.2.3). Explanation of the underlying pathogenesis of DIV is at a rudimentary level. In general terms, a drug, perhaps in hapten form, provokes an immune response leading to deposition of immune complexes of antibody, complement, and fibrin on the walls of blood vessels together with the release of anaphylatoxins C3a and C5a that recruit or ultimately trigger inflammatory cells and mediators, adhesion molecules, TNF, IFNγ, and a number of cytokines, particularly IL-1β, IL-2, IL-6, and IL-8 (CXCL8). These processes of an initial induced antibody response, immune complex formation, cell and mediator recruitment, and deposition within vessels produce vascular tissue damage. In ANCA-associated vasculitis, the destruction of neutrophils may activate inflammatory cells triggering proinflammatory cytokines, release of reactive oxygen species, and ultimately blood vessel wall destruction.

### 3.12.3 Drug-Induced Lung Diseases

Hypersensitivity reactions are one of several different mechanisms producing drug-induced lung disease. These reactions result from interaction of drug with the immune system and involve drug-specific antibodies and/or, more usually, drug-specific T cells. Eosinophilic pneumonia can be caused by almost any medication, while reports of drug-induced hypersensitivity pneumonitis, a combined type III and IV reaction in a Th1/Th17 response, are increasing, particularly to antineoplastic drugs (see Chap. 14, Sect. 14.2.2.3 and Chap. 15, Sects. 15.2.3.3, 15.2.5.3 and 15.3.2.6).

### 3.13 Drug-Induced Pruritus

For a summary of some important but different itch-inducing drugs, see Chap. 2, Sect. 2.5.12. An understanding of the pathophysiology of pruritus is substantially lacking, especially at the subcellular level in identifying and defining the roles of inflammatory and signaling molecules. Pruritus is associated with many hypersensitivity reactions but may have a number of different causes and underlying mechanisms rather than fitting neatly into any of the four hypersensitivity types. Itch is broadly classified as histaminergic or non-histaminergic. Vancomycin is a well-known histamine releaser via a non-immunological process from human mast cells and basophils (Chap. 6, Sect. 6.1.4.2). Red man syndrome (see Fig. 6.5) is a consequence of the release of this mediator, and the severity of the resultant erythema and pruritus is proportional to the concentration of the released histamine. For information on the recently identified relationship between vancomycin-induced red man syndrome, Staphylococcus aureus delta toxin-induced erythema and itch, the human Mas-related G protein receptor X2 receptor MRGPRX2, and mast cell degranulation, see Chap. 6, Sect. 6.1.4.2.

Several mechanisms have been suggested for chloroquine-induced pruritus including genetics (since black African background seems to be strongly associated), a consequence (not specified) of antecedent malaria infections and parasite density in the blood, slower metabolism of the drug, and, more recently, the involvement of “itch receptors,” Mrgprs (Mas-related G-protein-coupled receptors) (Sect. 3.2.5), a family of G-protein-coupled receptors expressed exclusively in peripheral sensory neurons. Studies in mice showed that less chloroquine-induced itch, but not itch induced by histamine, resulted in mice lacking a cluster of Mrgpr genes and the drug excites sensory neurons activating mouse MrgprA3 and human MrgprX1. Intradermal
injection of BAM8–22 induces itch in mice and humans, an itch that is not relieved by antihistamines. It is believed that BAM8–22 (bovine adrenal medulla peptide 8–22) may be an endogenous itch mediator in humans acting through MrgprX1, and the receptor may therefore be a potential therapeutic target.

Opioid-induced itching is centrally mediated via μ opioid receptors with the medullary dorsal horn being the site of action although serotonin and dopamine receptors may also be involved. Pharmacological evidence reinforces the belief of μ opioid receptor involvement, in particular, the effectiveness of the μ receptor antagonist naloxone in treating intrathecal and epidural opioid-induced itch. Other suggested mechanisms include the direct histamine-releasing effect of some opioids and, presumably on the basis that some opioids are serotonin reuptake inhibitors, modulation of serotonergic pathways (e.g., use of 5-HT3 receptor antagonists ondansetron, granisetron, and dolasetron).

Quite unlike the other agents inducing pruritus, hydroxyethyl starch (HES) may provoke itch by mechanically irritating nerve endings. HES does not cause degranulation of mast cells or release histamine from mast cells and basophils, nor does it induce release of substance P from macrophages and relief does not eventuate following antihistamine, corticosteroid, neuroleptic, and a variety of other therapies. More than 25 years ago, deposits of HES were found in lysosomal deposits in the skin, mainly in macrophages, endoneural macrophages of nerve fascicles, and keratinocytes and Langerhans cells. The amount of HES stored in lysosomes correlated with both the amount infused and the interval between infusions. HES deposits in nerves persisted for up to about 17 months which paralleled the cessation of pruritus. Deposits were also found in organs including liver and spleen, amounts deposited were greater in those with pruritus, and decreases occurred with time. Deposits were seen in Schwann cells of cutaneous nerves and in small peripheral nerves in those suffering from pruritus.

Pruritus is seen commonly after many targeted cancer treatments (overall incidence 17.4%), with or without a rash, and often early after the initiation of therapy. Targeted therapies known to provoke pruritus include epidermal growth factor inhibitors, HER2 inhibitors, vascular endothelial growth factor receptor inhibitors, tyrosine- and RAF-kinase inhibitors, mTOR inhibitors, and some mAbs such as those targeted to CD20 and CTLA-4. Although information on the mechanisms of action of the targeted drugs is generally available in some (but often still inadequate) detail (see Chap. 14), mechanisms underlying the drug-induced reactions are usually incomplete.

3.14 Summary

• For many drugs it has not been possible to explain allergic reactions on the basis of their chemical reactivity, protein-binding capacity, their biotransformed or degradative products, or the presence of a reactive impurity.

• Some allergic responses, sometimes even life-threatening as with anaphylaxis, occur on first exposure to a drug.

• There is at least one group of drugs, the neuromuscular blockers (and probably more to be identified), that can specifically elicit antibody-induced mast cell activation and release without first undergoing coupling to a macromolecular carrier. For these drugs, the di- or multivalency which is an inherent part of the molecular structure initiates mediator release by cross-linking cell-bound antibodies.

• An early clue to specific immune recognition of “small,” unbound chemicals, and hence drugs, was the demonstration of selective interaction of nickel ions with an MHC-II-bound peptide.

• In the production of IgE antibodies, antigen (usually in peptide form) is presented to T cells via MHC molecules on dendritic cells. The T cells undergo activation and clonal expansion. Antigen presentation to activated T cells by activated B cells ultimately results in co-stimulation of the B cells, class switching, clonal expansion, and differentiation to effector cells.

• The IgE and IgG molecules show two obvious differences: 6 domains (a dimer of Ce2-
Ce3-Ce4) that make up the Fc piece of IgE compared to 4 domains (a dimer of Cγ2-Cγ3) of the IgG Fc piece and the absence of a hinge joining the IgE H chains.

• Omalizumab, a recombinant humanized IgG1k monoclonal antibody, is used for patients with difficult-to-manage severe persistent allergic asthma. It binds to the Ce3 region of circulating human IgE antibodies inhibiting their binding to the FceRI and FcεRII receptors and thus ultimately suppressing IgE-mediated mast cell activation and the allergic inflammatory response. It does not target receptor-bound IgE on mast cells and thus does not trigger mast cell degranulation.

• The initial event in the activation of mast cells for mediator release is the binding of IgE antibodies to the high affinity (K, 10−10 M) FcεRI IgE receptor abundantly expressed on the mast cell and basophil surfaces.

• A second receptor for IgE, the low affinity receptor FcεRII also known as CD23, augments humoral and cellular responses. It is expressed on airway smooth muscle cells and several types of hematopoietic cells including mature B lymphocytes, macrophages, monocytes, dendritic cells, and eosinophils.

• The critical initial event for mediator release is the cross-linking by allergen molecules of IgE antibodies bound to the α chain of FcεRI. The capacity of the Ce2 domains of IgE to twist and bend makes the IgE molecule highly flexible for antigen binding and cross-linking of receptors.

• Cross-linking of receptors causes their aggregation, rapid migration to lipid rafts, activation of the Lyn and Fyn protein tyrosine kinases, and ultimately transphosphorylation of the β and γ chains and involvement of the Syk kinase.

• Released preformed mediators of inflammation and anaphylaxis stored in the cytoplasmic granules of mast cells include histamine and serotonin; proteoglycans heparin and chondroitin sulfate; serine and other proteases, mainly tryptase, chymase, cathepsin-G, and carboxypeptidase; lysosomal enzymes β-glucuronidase and β-hexosaminidase; cytokines including TNF, IL-4, stem cell factor, and fibroblast growth factor; and eosinophil, neutrophil, and monocyte chemotactic factors.

• The newly synthesized group of released mediators includes prostaglandin D2 (PGD2); thromboxanes; leukotrienes LTβ3, LTβ4, and LTD4; and platelet activating factor (PAF).

• Human mast cells can be activated by a number of different receptors in addition to the FcεRI and FcεRII receptors. The mast cell receptor, mastocyte-related G-protein-coupled receptor member X2 or MRGPRX2, has been identified as a target for several ligands including the neuropeptides substance P, vasoactive intestinal polypeptide, and somatostatin.

• Discovery of MRGPRX2 and its murine ortholog Mrgprb2 identifies the receptor as a trigger for so-called pseudoallergic and neurogenic reactions that occur independently of the FcεRI degranulation pathway.

• Histamine is synthesized from L-histidine by the inducible enzyme L-histidine decarboxylase and inactivated by histamine N-methyltransferase-catalyzed methylation of the imidazole ring and oxidative deamination of the primary amino group catalyzed by diamine oxidase.

• The physiological and pharmacological effects of histamine are mediated through four different receptors H1, H2, H3, and H4, all members of the seven-transmembrane G-protein-coupled receptor (GPCR) family with amino terminal glycosylation sites and phosphorylation sites for protein kinases A and C.

• Pathophysiological effects resulting from stimulation of the H1 receptor include those responses seen in immediate allergic reactions, viz., redness, itch, swelling, asthma, anaphylaxis, bronchoconstriction, and vascular permeability.

• H2 receptors appear to mainly mediate suppressive activities of histamine including gastric acid secretion, heart contraction, cell proliferation, differentiation, and some effects on the immune response.
• The H₃ receptor regulates the synthesis and release of histamine and also has a regulatory role in the release of neurotransmitters such as serotonin, dopamine, and norepinephrine.

• The H₄ receptor is functionally expressed on mast cells, eosinophils, monocytes, dendritic cells, and CD8+T cells. The receptor exerts a chemotactic effect on several cell types associated with immune and inflammatory responses such as allergy, asthma, rheumatoid arthritis, and inflammatory bowel disease.

• LTC₄ and LTD₄ are powerful mediators of asthma, airway hypersensitivity, and allergies inducing bronchoconstriction, increasing vascular permeability, and promoting mucous secretion. LTE₄ is present in greatest amount in vivo where it induces bronchial eosinophilia and airway hyperresponsiveness. The bronchoconstriction provoked by LTE₄ is strong in patients with aspirin-sensitive asthma but much weaker in other asthmatics. LTD₄ is much more pronounced in asthmatic patients not sensitive to aspirin.

• Cysteinyl leukotrienes are generated de novo from arachidonic acid by phospholipase A2 with the initial participation of 5-lipoxygenase-activating protein and the enzyme 5-lipoxygenase.

• The two human cysteinyl leukotriene receptors CysLT₁R and CysLT₂R do not bind the three cysteinyl leukotriene ligands equally: for CysLT₁R, LTD₄ > LTC₄ > LTE₄; for CysLT₂R, LTC₄ = LTD₄ > LTE₄.

• By screening cDNAs from the P2Y receptor family, the oxoglutarate receptor (Oxgr1) GPR99 was implicated as a potential third leukotriene receptor. CysLT₃R/GPR99 has high affinity for LTC₄ and is predominately an epithelial cell receptor.

• PAF, unlike histamine and tryptase, showed a good correlation with severity scores when these mediators were measured in blood from patients during acute allergic reactions.

• In mice, relatively large concentrations of IgG antibodies and antigen, plus neutrophils, macrophages, and basophils, as well as mast cells, with FcγRI, are known to induce anaphylaxis. In addition to the classical pathway mediated by FcεRI, mast cells, histamine, and PAF, anaphylaxis can occur in mice via an IgG-FcγRIII-macrophage-PAF pathway. There is, as yet, no conclusive evidence for an IgG antibody-mediated mechanism in humans.

• Recruitment of the Syk kinase and subsequent phosphorylation activation steps involving Lyn lead to mast cell activation demonstrating the importance of protein tyrosine kinases in the pathways that result in allergic inflammation and anaphylaxis.

• Sphingosine-1-phosphate, a major regulator of the vascular system and B and T cell trafficking, is elevated in the lungs of asthmatics where it regulates pulmonary epithelium permeability and is thought to contribute to the pathogenesis of anaphylaxis and rheumatoid arthritis.

• Urticaria is a heterogeneous disease with many subtypes caused by a range of agents and stimuli. Subtypes include urticaria due to genetic or immune mechanisms, urticaria with an autoimmune basis, and non-immune-mediated urticaria and angioedema.

• The combination of actions of ACE inhibitors of decreasing angiotensin II and aldosterone and increasing and maintaining bradykinin levels may lead to fluid extravasation into subcutaneous tissue ultimately producing angioedema.

• Angioedema may also occur following administration of angiotensin II receptor binding inhibitors such as losartan.

• The allergen-induced late-phase reaction has features of a delayed type IV cell-mediated hypersensitivity response but shows some significant differences best illustrated by the different cytokine profiles.
• Drug-induced delayed-type cutaneous hypersensitivity reactions manifest mainly as exanthemas, mediated by CD4+ and CD8+ CD3+ T cells in the dermis and epidermis. There are two phases of the hypersensitivity response, sensitization (or initiation or induction) involving keratinocytes, Langerhans, and dendritic cells and elicitation via T cells.

• T effector cells Th17, induced from naïve T cells by the cytokines TGF-β and IL-6 and enhanced by IL-23, are characterized by expression of distinct transcription factors RORγT, STAT3, and IRF-4 and the production of proinflammatory molecules of the IL-17 family comprising IL-17A, B, C, D, E, and F.

• IL-17A gives rise to tissue inflammation by producing proinflammatory cytokines IL-6 and TNF and chemokines CCL2, CXCL1, and CXCL2 that activate macrophages and granulocytes.

• Allergic contact dermatitis is a Th1 and CD8+ T cell-mediated disease. Ni allergy, involving activation of HLA-restricted, skin-homing Ni-specific T cells by antigen-presenting cells, is perhaps the best-known commonly occurring form. Both sensitization and skin reactions to Ni are thought to be mediated by CD4+ and CD8+ effector T cells producing IFN-γ.

• CD4+ and CD8+ T cells are found in the skin and blood of patients with maculopapular exanthema. Perforin-positive T cells have been found in patients and CD4+ T cells eluted from patch tests after drug-induced reactions.

• Mediated by activated CD8+ T cells, fixed drug eruption is a disease instigated by drugs in more than 95% of cases. CD8+ T cells activated via the CD3-TCR complex secrete IFN-γ in high amount as well as TNF, perforin, granzyme B, and Fas ligand (FasL).

• Immunohistological investigations of symmetrical drug-related intertriginous and flexural exanthema (SDRIFE; baboon syndrome) showed that most perivascular T cells were CD4+. Histology of a positive skin test in a patient with SDRIFE revealed infiltration of the dermis with mainly CD3+ and CD4+ cells and a few CD8+, CD20, and CD56 cells, findings compatible with a T cell-mediated hypersensitivity.

• The mechanisms underlying psoriasis can be viewed as three steps: keratinocytes activate dendritic cells via presented antigen and the production of cytokines, cathelicidin, and LL37/self RNA; and dendritic cells stimulate differentiation and activation of T cells producing IL-17; activated T cells secrete cytokines IL-17A, IL-17F, IL-22, and IFN-γ that activate keratinocytes which produce antimicrobial peptides and proinflammatory mediators.

• In acute generalized exanthematous pustulosis (AGEP), drug-activated dendritic cells lead to activation and expansion of drug-specific CD4 and CD8 T cells which migrate to the skin where they cause apoptosis of keratinocytes and formation of subcorneal vesicles. Cell death is mediated by the cytotoxic proteins granzyme B, perforin, granulysin and Fas/Fas ligand.

• Some HLA variants are known to be associated with drug reaction with eosinophilia and systemic symptoms (DRESS) in certain populations, for example, carbamazepine with HLA-B*15:02 in Southeast Asian populations and with HLA-B*31:01 in European populations.

• Many investigators believe that a concomitant human herpes virus 6 (HHV-6) reactivation with hypogammaglobulinemia caused by the drug is associated with the DRESS hypersensitivity syndrome.

• Activated T cells produce TNF, IFNγ, and IL-2, mediators that may produce the symptoms seen in DRESS patients. Skin biopsies and patch tests show positive results for perforin, granzyme B, FasL, IFNγ, and IL-5.

• Continued production of CD8+ T cell clones may be responsible for sustaining secondary tissue injury, and fluctuations in the CD8+ repertoire suggest that newly formed clones may have a role in the maintenance of DRESS injury.

• Eosinophil infiltration is pronounced in DRESS, being found in 80% of diagnosed patients. IL-5, a key cytokine for eosinophil
recruitment, proliferation, and activation, along with eotaxin-1 (CCL11) and thymus activation-regulated chemokine (TARC, CCL17), acts to recruit eosinophils in drug-induced cutaneous eruptions as seen in DRESS.

- As in DRESS, drug-induced liver injury is associated with fever, rash, eosinophilia, and liver infiltrates, and the reaction is generally a type IV hypersensitivity response involving CD4+ cells and CD8+ cytotoxic lymphocytes.
- Granulysin appears to be a key toxic molecule responsible for disseminated keratinocyte killing in TEN/SJS. TNF appears to be the upstream regulator of granulysin, the key mediator of keratinocyte detachment in the diseases.
- In a recent large randomized controlled trial, etanercept targeted to TNF was compared to the traditional corticosteroid therapy. Etanercept significantly decreased the predicted mortality rate and reduced skin healing time. Findings provide a rationale for the clinical use of anti-TNF biologic agents (including mAbs such as infliximab) in the treatment TEN/SJS.
- Skin depigmentation in vitiligo seems to be the result of an autoimmune process together with the actions of cytokines such as interferons, IL-2, sIL-2R, IL-10, IL-13, and IL-17A and CD8+ cytotoxic T lymphocytes targeting melanocytes and expressing IFNγ. Blocking NLRP3 inflammasome activation in keratinocytes inhibits production of cytokines by inhibiting chemotaxis of CD8+ T cells from vitiligo patients.
- Increased levels of Th1 cytokines IFNγ, IL-1α, IL-1β, and IL-2 with decreased numbers of Th2 helper cells in the dermis are thought to be associated with neutrophil activation and localization in Sweet’s syndrome (SS). TNF, IFNγ, IL-17, and IL-1β together produce an inflammatory response and recruit and localize neutrophils by inducing adhesion molecules and the chemoattractant CXCL8.
- Pruritus is associated with many hypersensitivity reactions but may have a number of different causes and underlying mechanisms rather than fitting neatly into any of the four hypersensitivity types.
- Itch is broadly classified as histaminergic or nonhistaminergic. An understanding of the pathophysiology of pruritus is substantially lacking, especially at the subcellular level in identifying and defining the roles of inflammatory and signaling molecules.
- Studies in mice showed that less chloroquine-induced itch, but not itch induced by histamine, resulted in mice lacking a cluster of Mrgrp genes and the drug excites sensory neurons activating mouse MrgrpA3 and human MrgrpX1. BAM8–22 (bovine adrenal medulla peptide 8–22) may be an endogenous itch mediator in humans acting through MrgrpX1, and the receptor may be a potential therapeutic target.
- Abacavir-HLA binding studies indicate that the drug changes the shape of the antigen-binding cleft. This results in preferred binding of smaller amino acids, an alteration in the repertoire of self-peptides that bind HLA-B*57:01 and a T cell response to self-proteins presented only in the presence of abacavir.
- Carbamazepine, a drug strongly associated with HLA-B*15:02, binds to this allotype and alters the repertoire of presented self-peptides. The most likely binding site on the carbamazepine molecule is the ketone of its 5-carboxamide group on the tricyclic ring.
- The mast cell and possibly the basophil appear to be the cells most likely involved in the desensitization of patients to type 1 immediate drug reactions. FcεRI-bound antigen-specific IgE molecules do not disappear from the cell surface during desensitization after becoming bound to small doses of antigen.
- Basophils from the nonreleaser phenotype lack detectable Syk, a protein tyrosine kinase involved in the initiation of FcεRI-mediated secretion. FcεRI on human skin mast cells becomes nonresponsive with antigen concentrations that do not induce release. As with basophils, this nonresponsive state correlates with reduced Syk levels.
- Desensitization of cell-mediated cutaneous reactions is even less well understood than desensitization of immediate allergic...
reactions, and there are doubts about its efficacy and safety. Currently, the procedure tends to be restricted to uncomplicated exanthems without systemic symptoms and fixed drug eruptions.

- It is generally accepted that culprit drugs involved in the severe and life-threatening delayed hypersensitivity reactions SJS and TEN but also DRESS (DIHS), and perhaps even AGEP, are contraindicated for desensitization.

- Successful, or partly successful, desensitizations have been undertaken with TMP-SMX, oxcarbazepine, allopurinol, and some antibiotics used for cystic fibrosis.

- Examples of type II cytotoxic antibody-mediated drug reactions include drug-induced immune hemolytic anemia, drug-induced thrombocytopenia where a number of different mechanisms are involved, and acute agranulocytosis in which more than 70% of cases are caused by drugs.

- Type III drug-induced hypersensitivities, that is, antigen-antibody complex-mediated reactions, occur in some cases that closely resemble classical serum sickness. Many drugs have been implicated including β-lactams, sulfonamides, ciprofloxacin, tetracycline, NSAIDs, carbamazepine, allopurinol, thiouracil, and monoclonal antibodies. Circulating antigen-antibody complexes are formed after drugs become protein-bound in vivo and stimulate IgG and/or IgM antibodies. The liberation of vasoactive amines is thought to play a part in tissue deposition.

- Hypersensitivity vasculitis induced by drugs is another manifestation of a type III response. Many drugs have been implicated including some β-lactams, particularly, amoxicillin and cephalaxin, cotrimoxazole, and NSAIDs.

- In vasculitis, formation of immune complexes of drug, antibody, complement, and fibrin on the walls of blood vessels together with the release of anaphylatoxins C3a and C5a leads to recruitment and triggering of inflammatory cells and mediators, adhesion molecules, TNF, IFNγ, and a number of cytokines, particularly IL-1β, IL-2, IL-6, and IL-8 CXCL8. Deposition of complexes within vessels produces vascular tissue damage.

- A proportion of small-vessel vasculitis patients have drug-induced anti-neutrophil cytoplasmic antibodies (ANCA). In ANCA-associated vasculitis, the destruction of neutrophils may activate inflammatory cells triggering proinflammatory cytokines and ultimately blood vessel wall destruction.

- Hypersensitivity reactions are one of a number of different mechanisms producing drug-induced lung disease. Eosinophilic pneumonia can be caused by almost any medication, while reports of drug-induced hypersensitivity pneumonitis, a combined type III and IV reaction in a Th1/Th17 response, are increasing, particularly to antineoplastic drugs.

Further Reading

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