CHAPTER 9

IMMUNOREGULATION BY NATURALLY OCCURRING AND DISEASE-ASSOCIATED AUTOANTIBODIES:

Binding to Cytokines and Their Role in Regulation of T-Cell Responses

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Abstract: The role of naturally occurring autoantibodies (NAbs) in homeostasis and in disease manifestations is poorly understood. In the present chapter, we review how NAbs may interfere with the cytokine network and how NAbs, through formation of complement-activating immune complexes with soluble self-antigens, may promote the uptake and presentation of self-molecules by antigen-presenting cells. Both naturally occurring and disease-associated autoantibodies against a variety of cytokines have been reported, including NAbs against interleukin (IL)-1α, IL-6, IL-8, IL-10, granulocyte-macrophage colony-stimulating factor, interferon (IFN)-α, IFN-β, IFN-γ, macrophage chemotactic protein-1 and IL-21. NAbs against a variety of other self-antigens have also been reported, and using thyroglobulin as an example we discuss how NAbs are capable of promoting uptake of immune complexes via complement receptors and Fc-receptors on antigen-presenting cells and thereby regulate T-cell activity. Knowledge of the influence of NAbs against cytokines on immune homeostasis is likely to have wide-ranging implications both in understanding pathogenesis and in treatment of many immunoinflammatory disorders, including a number of autoimmune and autoinflammatory diseases.
INTRODUCTION

Naturally occurring antibodies play an essential role in our defense against invading microorganisms by directly neutralizing viruses and bacteria, by activating the complement system and by enhancement of phagocytosis, as described elsewhere in this book and in reference 1. In contrast, naturally occurring autoantibodies (NAbs) are characterized by binding to self-molecules, and their primary function may involve clearance of senescent cells and metabolic waste products, but they also appear to play important roles in immunoinflammatory processes.2-6 In the present chapter, we shall focus on the immunomodulatory effects that self-reactive NAbs may have as a result of interactions with cytokines or formation of complement-activating immune complexes (ICs) with other soluble self-antigens and targeting these self-antigens to B cells and presumably other antigen-presenting cells (APCs).

NAbs have been shown to bind a variety of self-molecules, ranging from cytokines and other plasma proteins to tissue-specific antigens, structural proteins, metabolic enzymes, heat shock proteins and DNA (Tables 1 and 2). Some NAbs, including those reacting with various cytokines and thyroglobulin (TG), are relatively specific, while others, e.g., IgM anti-DNA antibodies of all species, tend to be polyreactive.7 NAbs exist as IgM, IgA and IgG isotypes. The occurrence of the IgG isotype among NAbs is indicative of T-cell involvement in shaping of the autoreactive B-cell repertoire.8-10 IgM NAbs are already synthesized in newborns, and different babies produce IgM NAbs to a similar set of self-molecules.10

AUTOANTIBODIES AGAINST CYTOKINES

Several reports have documented the presence of autoantibodies against cytokines both in healthy individuals (NAbs) and in patients with various immunoinflammatory disorders (disease-associated autoantibodies, DAbs) (Table 1). In most cases, the physiological and/or pathological significance of these antibodies is unclear. However, recent evidence suggests that certain anti-cytokine NAbs have significant, if not decisive, pathogenic roles in rare disorders (reviewed in refs. 9, 11, 12).

Naturally Occurring Autoantibodies against Cytokines

The biological roles of NAbs against cytokines are poorly understood. Fab fragments of some of these NAbs bind in a saturable and highly specific manner to their respective cytokine. as for example in the cases of NAbs to IL-1α, IL-6 and GM-CSF.9 In some cases, these antibodies are found at levels at or above 50 nM, for example in healthy blood donors and apparently without untoward effects.13

Why and how high-affinity NAbs to some cytokines and not to others are induced in healthy individuals is unknown. Also obscure is whether these in vitro neutralizing antibodies neutralize their respective cytokines in vivo too, or whether they exhibit carrier- or cytokine-regulatory, or even cytokine-protective functions.9
**Table 1.** Selected cytokines to which naturally occurring autoantibodies have been reported

| NAbs against Cytokine | DAbs against Cytokine | Disease Association |
|-----------------------|-----------------------|--------------------|
| **Type 1 interferons**| **Type 1 interferons**|                    |
| IFN-α/β               | IFN-α/β/ω             | Viral infections, autoimmune and neoplastic diseases, chronic graft-vs.-host disease, thymoma, myasthenia gravis, autoimmune polyendocrinopathy syndrome Type I |
| **Type 2 interferons**| **Type 2 interferons**|                    |
| IFN-γ                 | IFN-γ                 | Viral and mycobacterial infections, multiple sclerosis, Guillain Barré syndrome |
| **Interleukins**      | **Interleukins**      |                    |
| IL-1α                 | IL-1α                 | Rheumatoid arthritis, juvenile chronic arthritis, thymoma, myasthenia gravis |
| IL-2                  | IL-2                  | inflammatory bowel diseases |
| IL-3                  |                       |                    |
| IL-6                  | IL-6                  | Rheumatoid, arthritis, systemic sclerosis, alcoholic cirrhosis, Type 2 diabetes |
| IL-10                 | IL-10                 | Inflammatory bowel diseases |
|                       | IL-12p35 and IL-12p40 | Thymoma, myasthenia gravis |
|                       | IL-17A, IL-17F        | Thymoma, myasthenia gravis, autoimmune polyendocrinopathy syndrome Type I |
| IL-21                 | IL-22                 | Autoimmune polyendocrinopathy syndrome Type I |
| **Growth factors**    | **Growth factors**    |                    |
| GM-CSF                | GM-CSF                | Pulmonary alveolar proteinosis |
| G-CSF                 | G-CSF                 | Felty’s syndrome (rheumatoid arthritis), systemic lupus erythematosus |
| TGF-β                 | TGF-β                 | Inflammatory bowel diseases |
| NGF                   |                       |                    |
| **Other cytokines**   | **Other cytokines**   |                    |
| LIF                   |                       |                    |
| TNF-α                 |                       |                    |
| **Chemokines**        | **Chemokines**        |                    |
| IL-8                  | IL-8                  | Acute lung injury/acute respiratory distress syndrome |
| MCP-1                 | MIP-α/β               | HIV infection |

1Abbreviations: NAbs: naturally occurring autoantibodies; DAbs: disease-associated autoantibodies; IFN: interferon; IL: interleukin; GM-CSF: granulocyte-macrophage colony-stimulating factor; G-CSF: granulocyte colony-stimulating factor; TGF: transforming growth factor; NGF: nerve growth factor; LIF: leukemia inhibitory factor; TNF: tumor necrosis factor; MCP: monocyte chemotactic protein; MIP: macrophage inflammatory protein.
IMMUNOREGULATION BY NAbs AND DAbs

NAbs against Type 1 and Type 2 Interferons

Low levels of IgG and IgM capable of neutralizing IFN-α, IFN-β and IFN-γ have been detected in blood of apparently healthy individuals (as reviewed in refs. 14, 15). IgG NAbS against IFN-α are especially frequent, and are easily detectable in pharmaceutical preparations of normal human IgG (IVIG). NAbS against IFN-α have been found in approximately 10% of healthy Caucasians, but the exact frequency is likely to be higher, as these NAbS are often difficult to detect in plasma because they are complexed with native IFN-α. IgG NAbS against IFN-α are of high avidity and specific, as they do not cross-bind IFN-β or other cytokines. NAbS to IFN-α have been demonstrated in vivo in bioactive form after treatment with IVIG. As these NAbS neutralize IFN-α, IVIG therapy suppresses both antiviral and other effects of endogenous IFN-α, which may explain some of the many therapeutic effects of IVIG.

NAbS against Interleukin-1α

NAbS to interleukin (IL)-1α were first found by direct binding of IgG from normal individuals to radiolabeled, human, recombinant IL-1α and by IgG-mediated competitive

Table 2. Selected self-antigens to which naturally occurring autoantibodies have been reported

| Antigen                          | Reference(s) |
|---------------------------------|--------------|
| Acetylcholinesterase10           |              |
| Actin8,76,87-89                  |              |
| Albumin10,87-89                  |              |
| Anion transport protein (band 3)7 |              |
| Annexin10                       |              |
| Beta-galactosidaseγ              |              |
| Beta2-crystallin10               |              |
| Beta2-microglobulin10            |              |
| Cardiolipin91                    |              |
| Catalase10                       |              |
| CD476                           |              |
| Chorionic gonadotropin10         |              |
| Collagen88,92                    |              |
| Cytochrome c88                   |              |
| dsDNA8,10,76,89,93,94            |              |
| Factor II10                      |              |
| Factor VIII95                    |              |
| Factor X10                       |              |
| Fetuin87                        |              |
| Fibrin10                         |              |
| Fibrinogen10                     |              |
| GAD10                           |              |
| Galectin 1 and -310             |              |
| Geisolin10                       |              |
| Hemoglobin-α10                   |              |
| HLA class I96                   |              |
| Hsp4010                          |              |
| Hsp4γ10                         |              |
| Hsp60 peptides10                 |              |
| Hsp9097                         |              |
| IgG91                            |              |
| Insulin90                        |              |
| Keratin8,10,88                   |              |
| Laminin94                        |              |
| LDL10                           |              |
| MOG10                           |              |
| Myelin basic protein65,89        |              |
| Myoglobin8,76,87-89,94           |              |
| Myosin10,89,94                   |              |
| Prolactin89                      |              |
| Protease10                       |              |
| Pyruvate dehydrogenase91         |              |
| Spectrin93                       |              |
| ssDNA10,91                       |              |
| T-cell receptors98               |              |
| Thyroglobulin16,58,76,77,79,80,87,89,94,99 | |
| Thyroid peroxidase84,89,100      |              |
| Transferrin8,76,87,88            |              |
| Tubulin8,87,89,93                |              |
| Ubiquitin10                      |              |

The table is not complete with respect to reported antigens and references.
interference with IL-1α binding to cellular IL-1 receptors. Subsequently, human IVIG, cord blood and sera of patients with various immunoinflammatory disorders were found to contain high-avidity autoantibodies that bind to and neutralize IL-1α both in vitro and in vivo. They bind IL-1α in a saturable fashion and through the Fab fragments of the IgG isotypes IgG1, IgG2 and IgG4. The occurrence of detectable anti-IL-1α IgG in sera of healthy individuals vary with age and sex with male preponderance and markedly increased frequency with age (up to 75% positives among elderly males).

A human anti-IL-1α autoantibody has been cloned. It is an IgG4/k monoclonal antibody which reacts with IL-1α, but not with IL-1β, the IL-1-receptor antagonist (IL-1Ra) or several other cytokines. It binds with high affinity (Kd ≈ 10^(-10) M), and the presence of somatic mutations in the variable regions suggests antigen-driven affinity maturation.

As IL-1α from antigen presenting cells is an important co-activator of T cells, particularly in its membrane-bound form, it is important to note that anti-IL-1α NAbs not only neutralize IL-1α in lysates of human blood monocytes, but also membrane-associated IL-1-α activity.

NAbs against Interleukin-6

IgG NAbs to IL-6 were first reported in sera of normal individuals. Since then, the presence of NAbs to IL-6 and similar antibodies in patients with immunoinflammatory and fibrotic diseases has been confirmed (reviewed in refs. 9, 11, 14, 15). High-affinity IgG NAbs to IL-6 have been detected in up to 15% of normal Danish blood donors with 1% having titers ranging from 64 to greater than 10,000 and 0.1% having exceedingly high titers. The anti-IL-6 NAbs bind to IL-6 through their Fab fragments and they effectively inhibit binding of IL-6 to IL-6 receptors and, hence, neutralize the bioactivity of IL-6. NAb-positive donors with high antibody titers have no overt signs of pathology even though they are likely to be functionally IL-6-deficient. NAbs to IL-6 are detectable in IVIG and are found in bioactive form, binding IL-6 in the circulation following IVIG administration.

NAbs against Interleukin-10

IgG NAbs against IL-10 have been reported in normal sera and in preparations of pooled normal human IgG. Indeed, 0.4% of healthy Danish blood donors present with these NAbs at such high concentrations and avidity that these blood donors are functionally IL-10-deficient. These NAbs are of the IgG isotype and of polyclonal origin. They prevent IL-10 from binding to its receptor thereby neutralizing IL-10 bioactivity. Anti-IL-10 NAbs are highly specific in that they fail to bind viral forms of IL-10 and other members of the human IL-10 family including IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29.

NAbs against Granulocyte-Macrophage Colony-Stimulating Factor

Using a direct radioligand binding assay, high-affinity NAbs to granulocyte-macrophage colony-stimulating factor (GM-CSF) were reported at very high titers in 4 of 1,238 (0.3%) apparently healthy blood donors. Later, all of 72 tested, apparently
healthy Japanese individuals were shown to possess low levels of these NAbs such that more than 99% of GM-CSF were bound and neutralized by these antibodies. Anti-GM-CSF NAbs have also been found in human IVIG.\textsuperscript{9,18}

NAb against XXC- and CC Chemokines

NAbs to various chemokines have also been reported. For example, IL-8- and macrophage chemotactic protein (MCP)-1-containing IgG-immune complexes have been demonstrated in sera from healthy individuals, where the chemokines themselves are usually not detected. It is hypothesized that circulating IgG NAbs to chemokines may play a role as a sink for the spill-over of chemokines produced in local tissues.\textsuperscript{28}

NAb against Other Cytokines

IgG NAbs to other cytokines, for example IL-2, IL-3, granulocyte colony-stimulating factor (G-CSF), nerve growth factor (NGF), leukemia inhibitory factor (LIF), tumor necrosis factor (TNF)-\(\alpha\), and soluble TNF receptors have also been reported in normal and diseased individuals, while IgE antibodies to IL-4, TNF-\(\alpha\), TNF-\(\beta\) and various chemokines have been reported in sera of AIDS patients. Some of both isotypes, however, cannot be regarded as NAbs in that they bind the relevant mediator(s) with low avidities and in some cases bind only to cytokines denatured by adsorption to nitrocellulose membranes or plastic surfaces (using ELISA-technologies).

Disease-Associated Autoantibodies against Cytokines (DAbs)

Anti-cytokine DAbs have been reported in a wide range of pathological disorders, suggesting that pathogenetically obscure diseases may eventually be at least partly explained by the presence of anti-cytokine DAbs (reviewed in refs. 9, 11, 12).

DAbs against Type 1 and Type 2 Interferons

Anti-Type 1 interferon (IFN) DAbs, preferentially of IgG type, were first reported in patients with varicella-zoster and hepatitis virus infections, in patients with autoimmune and neoplastic diseases, and in a patient with chronic graft-vs.-host disease (reviewed in refs. 15, 14, 15, 29, 30). Later reports have documented DAbs that bind to other IFN species, usually in patients with various infectious diseases or severe immunodeficiencies.

Neutralizing DAbs against IFN-\(\alpha/\beta/\gamma\) have been demonstrated primarily in patients with thymoma and/or myasthenia gravis.\textsuperscript{31} However, patients with thymic malignancy/myasthenia gravis have also been reported to express DAbs to a variety of other cytokines including, IL-1\(\alpha\), IL-12 p35 and IL-12p40, and IL-17A.\textsuperscript{31}

High-titer DAbs against IFN-\(\alpha2\) and IFN-\(\omega\) have been reported in 100% of European patients with autoimmune polyendocrinopathy syndrome Type I (APS-I).\textsuperscript{32} This disease is a result of mutations in the autoimmune regulator gene (AIRE), which impairs thymic self-tolerance induction in developing T cells. The ensuing autoimmunity particularly targets ectodermal and endocrine tissues, but chronic candidiasis is a frequent and early manifestation. Although the underlying immunodeficiency of APS-1 is unclear,
neutralizing anti-IFN DAbs and, most recently, anti-IL-17A and anti-IL-22 DAbs appear to be implicated directly in the pathogenesis, as they appear before development of candidiasis in all informative cases.\textsuperscript{33,34}

Anti-IFN-$\gamma$ DAbs have been reported in patients with viral infections and in cerebrospinal fluids from patients with multiple sclerosis and Guillain Barré syndrome (reviewed in ref. 15). Circulating DAbs to IFN-$\gamma$ have also been positively correlated with the severity of both tuberculous and nontuberculous mycobacterial infections (reviewed in ref. 11). It is characteristic that patients with extremely high antibody titers had rapidly progressive disease and severe immunodeficiency, most likely due to DAb-induced blockade of the crucial macrophage-activating effect of IFN-$\gamma$.

\textit{DAbs against Interleukin-1$\alpha$}

Although IL-1$\alpha$ is secreted from cells producing the cytokine, the dominant form of IL-1$\alpha$ appears to be the cell-associated precursor form that is found both intracellularly and on the surface of many cell types, including keratinocytes and ‘professional’ APCs such as macrophages and B cells. On these cells IL-1$\alpha$ is thought to be involved as a juxtacrine co-activator of T cells.\textsuperscript{9} IL-1$\alpha$ is usually absent in the circulation, if present then only at low concentrations. During infection and inflammation, however, substantial amounts of IL-1$\alpha$ may be found in the blood, most likely released from dying cells.

Cell-associated IL-1$\alpha$ is biologically active, and its biological activities are neutralized by antibodies to IL-1$\alpha$, including IgG anti-IL-1$\alpha$ NAbs, but not by antibodies against IL-1$\beta$.\textsuperscript{21}

The prevalence of DAbs to IL-1$\alpha$ in immunoinflammatory disorders vary considerably. For example, anti-IL-1$\alpha$ DAbs have been found more commonly and at higher levels in patients with non-destructive forms of arthritis.\textsuperscript{35} Progression of joint destruction in patients with rheumatoid arthritis was negatively associated with the occurrence of circulating IL-1$\alpha$ DAbs, but patients who seroconverted more than two years after the onset of RA showed the most aggressive development of joint erosion. Interestingly, transgenic mice overexpressing the membrane form of human IL-1$\alpha$ in macrophage-like and fibroblast-like synoviocytes develop severe arthritis, correlating with the degree of membrane expression of IL-1$\alpha$, but not circulating IL-1$\alpha$.\textsuperscript{36} Taken together, this suggests that IL-1$\alpha$ and/or the lack of IL-1$\alpha$ DAbs play a role in the erosive processes of rheumatoid arthritis. Along with DAbs to other cytokines, anti-IL-1$\alpha$ DAbs have also been demonstrated in patients with juvenile chronic arthritis, thymoma and myasthenia gravis.\textsuperscript{22,31}

\textit{DAbs against Interleukin-6}

There is an increased prevalence of high-avidity IgG DAbs to IL-6 in patients with rheumatoid arthritis and systemic sclerosis, and the presence of these antibodies signals a poor survival in patients with alcoholic cirrhosis, possibly because of an increased risk for recurrent infections.\textsuperscript{9,37,38} A 2.5-fold increase in anti-IL-6 DAb-positivity has recently been reported in Type 2 diabetic patients, and mice vaccinated with IL-6 develop obesity and impaired glucose tolerance.\textsuperscript{39} These data suggest that an autoimmune reaction against IL-6 may be involved in a subset of Type 2 diabetics.
**DAbs against Granulocyte-Macrophage Colony-Stimulating Factor**

Anti-GM-CSF DAbs are of clinical interest not only because of GM-CSF’s growth-potentiating effect on macrophages and granulocytes, but also because GM-CSF appears to be a central mediator affecting bronchial epithelial cells, possibly through its marked effect on eosinophils, both as a chemoattractant, growth promoter and stimulator. In accordance with results obtained in GM-CSF knockout mice, anti-GM-CSF DAbs have been associated with pulmonary alveolar proteinosis (PAP), a rare disease in which surfactant lipids and proteins accumulate in pulmonary alveolar macrophages and alveoli, resulting in respiratory insufficiency and failure. Recently, isolated anti-GMCSF DAbs from a patient with PAP were shown to reproduce the pathologic manifestations of the human disease in previously healthy primates. These findings may have therapeutic implications for the potential use of GM-CSF not only to treat PAP, but also other immunoinflammatory respiratory disorders such as asthma.

**DAbs against Granulocyte Colony-Stimulating Factor**

IgG DAbs to granulocyte colony-stimulating factor (G-CSF) have been demonstrated in Felty’s syndrome, a relatively rare complication in rheumatoid arthritis, and in some patients suffering from systemic lupus erythematosus (SLE) with accompanying neutropenia. IgM antibodies were found in 6 neutropenic and 3 normocytic SLE patients. Interestingly, anti-G-CSF antibodies were associated with an exaggerated serum level of G-CSF and a low neutrophil count. This may suggest that exposure to high levels of intrinsic G-CSF (not known whether bioinactive) may trigger the production of G-CSF DAbs and, further, that these DAbs may have a carrier function in vivo thus slowing the elimination of G-CSF from the circulation.

**DAbs against Other Cytokines**

Using radioimmune and radioreceptor assays, DAbs against macrophage-inhibitory protein (MIP)-1α and MIP-1β have been demonstrated in about 1% of patients, suffering from HIV infection. These antibodies specifically inhibited receptor binding of both chemokines; there was no association between the presence of antibodies and disease stage, or HIV progression rate.

IgG DAbs against IL-2, IL-10 and transforming growth factor-β (TGF-β) have recently been demonstrated at relatively high concentrations in up to 33% of patients with inflammatory bowel diseases using ELISA and Western blot assays. Neutralizing DAbs against IL-12 p35 and IL-12p40 have been demonstrated primarily in patients with thymoma and/or myasthenia gravis. Indeed, patients with thymic malignancy/myasthenia gravis express DAbs to a variety of other cytokines including IFN-α/β, IFN-α, IL-1α and IL-17A. Interestingly, among these patients those with opportunistic infections possess multiple anti-cytokine DAbs, suggesting that these antibodies may be important in the pathogenesis of infections in patients with thymic malignancy.

Most patients with autoimmune polyendocrine syndrome Type I suffer from chronic mucocutaneous candidiasis, and this immunodeficiency was recently associated with high titers of DAbs against IL-17A, IL-17F, and/or IL-22. The DAbs against IL-17A, IL-17F
and IL-22 neutralized these cytokines, but not a host of other cytokines. As these DAbs were not found in healthy controls nor in 102 patients with other immunoinflammatory disorders, DAbs against IL-17A, IL-17F and IL-22 may have a causative relationship with the development of chronic mucocutaneous candidiasis in patients with this polyendocrine syndrome.

Anti-IL-8 DAbs, often complexed with endogenous IL-8, have been shown to be an important prognostic indicator for the development and outcome of acute lung injury/acute respiratory distress syndrome (ARDS). The IL-8/anti-IL-8 complexes purified from lung edema fluids activate and trigger chemotaxis of neutrophils and regulate neutrophil apoptosis via IgG receptor FcγRIIa. These ICs promote an inflammatory phenotype of human umbilical vein endothelial cells, and they upregulate the expression of intercellular adhesion molecule (ICAM)-1 on the cell surface. Lung tissues from patients with ARDS also express high levels of ICAM-1. Hence, IL-8/anti-IL-8 complexes may contribute to pathogenesis of lung inflammation by inducing activation of endothelial cells through engagement of IgG receptors.

Therapy-Induced Autoantibodies to Cytokines (TAbs)

Anti-cytokine TAbs may develop in response to prolonged therapies with natural and recombinant-derived human cytokines. It is unclear whether preexisting anti-cytokine NAbs play a role in this regard, but they are probably not without importance, considering the high avidity and high titers of some of these NAbs (discussed above). In general, however, TAbs develop over time as a result of repeated ‘inoculations’ with cytokines. They may wax and wane, change in affinity, give rise to side-effects, and/or influence the primary intended therapy.

The development of neutralizing TAbs was first noted in scattered patients undergoing therapies with human fibroblast-derived IFN-β and human recombinant IFN-α. This finding received little attention at that time, but the increased use of recombinant human cytokines and cytokine constructs for therapeutic purposes have sharpened the awareness of the clinical importance of anti-cytokine TAbs. Thus, induction of TAbs has now been reported in patients treated with several human recombinant cytokines and growth factors including IFN-α, IFN-β, IFN-γ, IL-2, GM-CSF, often resulting in therapeutic failure and in rare cases even in serious side-effects (reviewed in refs. 9, 15, 55).

IMMUNOREGULATORY NAbs AGAINST NON-CYTOKINE SELF-ANTIGENS

While the above-mentioned anti-cytokine NAbs are immunoregulatory by nature, there is evidence to suggest that NAbs against other self-antigens may also be immunoregulatory, as we shall describe in the following.

NAbs against a broad variety of self-antigens has been demonstrated (Table 2), but their physiological functions, if any, has not been fully elucidated. While some of these NAbs have been suggested to play a role in the clearance of senescent cells and metabolic waste products, their role in maintenance (or breakage) of tolerance toward self remains obscure.

One mechanism by which NAbs against soluble self-antigens may play a regulatory role is by targeting self-antigens to APCs and thereby affecting self-antigen presentation.
to T cells. It is likely that NAbs of IgG isotype are capable of directing captured self-antigens to Fcγ-receptor expressing APCs. Similarly, NAbs of IgM or IgA isotype may direct self-antigens to B cells and macrophages, expressing the Fcα/μ receptor. All these events may promote presentation of self-antigens to autoreactive T cells.

One of the self-antigens against which NAbs have been most frequently described is thyroglobulin (TG). In a serum-free environment, a subset comprising 2–4% of normal, circulating B cells is capable of binding TG (this subset presumably represents B cells with polyreactive surface immunoglobulins), while TG binds to the entire B-cell population in the presence of autologous serum. The binding can be substantially inhibited by immunoabsorption of TG-reactive autoantibodies from serum or by heat inactivation of serum complement, suggesting that formation of complement-activating ICs promoted the binding of TG to the B cells. In accordance with this hypothesis, blockade of complement receptors 1 (CR1/CD35) and 2 (CR2/CD21) reduced the binding of TG to B cells by > 90%, and addition of TG to preparations of normal mononuclear cells suspended in sera from healthy individuals lead to increased deposition of C3-fragments on B cells. In accordance with these findings using TG as model self-antigen, Thornton et al. showed that normal serum contains NAbs with reactivity against the primary antigen keyhole limpet hemocyanin, and that ICs formed with these NAbs are capable of fixing fragments of complement component 3 (C3) and bind to B cells via CR1 and CR2 (Fig. 1B). They further demonstrated that expression of the T-cell co-stimulatory molecule CD80 by B cells required a secondary ligation of IC-associated IgG to FcγRII.

It is widely accepted that B cells bearing specific antigen-receptors efficiently take up and present antigens to T cells (Fig. 1A). As outlined, non-specific B cells may also engage in antigen presentation provided for the antigen in question is incorporated in complement-opsonized ICs and thereby targeted to CR1, CR2 and FcγRII (Fig. 1B). This recruitment of non-specific B cells increases the number of antigen-presenting B cells from a few antigen-specific ones to the entire B-cell population. However, only B cells bearing specific antigen receptors are eventually stimulated by T-helper (Th) cells for antibody production. Thornton et al. also showed that iC3b-containing TG/NAb complexes were also taken up by neutrophils, via complement receptors CR1 and CR3 (CD11b/CD18), indicating that the formation of ICs with NAbs may also be similarly important for the uptake of self-antigens by myeloid cells (of relevance for dendritic cells). Taken together, these findings suggest that antigen presentation is strongly promoted by incorporation of antigens into complement-activating ICs with NAbs. Correspondingly, the presentation of TG on B cells is proportional to the anti-TG NAb content of the surrounding serum, and is thus considerably higher in the presence of sera from patients with Hashimoto’s thyroiditis (HT) or Graves’ disease (GD) with a high content of anti-TG, as compared with sera from healthy individuals.

In analogous studies using myelin basic protein (MBP) as self-antigen, we found that sera from patients with multiple sclerosis (MS) and sera from healthy individuals contained approximately equal concentrations of MBP-reactive IgM. Upon addition of MBP to normal mononuclear cells suspended in patient or control sera, MBP co-deposited with IgM, IgG and C3-fragments on monocytes. While the deposition of IgM and C3 was approximately similar in patient and control sera, the IgG deposition was increased 9-fold in the presence of MS sera, presumably due to the presence of circulating high-affinity IgG DAbs in the patients. Notably,
the deposition of C3 fragments as well as that of IgM and MBP on monocytes was abrogated by disruption of the tertiary structure of MBP by boiling, as would be expected if complex formation depends upon the interaction of antibodies with conformational epitopes on MBP.
REGULATION OF T-CELL RESPONSES BY NAbs AND COMPLEMENT

In the presence of untreated serum, even CD4+ Th cells from healthy individuals respond to a challenge with TG at high concentrations (≥ 10 μg/ml), although TG induces increased responses by Th cells (and B cells) from patients with autoimmune thyroid disease. Inactivation of serum complement, immunoadsorption of TG-reactive NAbs or disruption of the tertiary structure of TG by boiling significantly inhibits the TG-induced Th cell proliferation and production of T-cell cytokines such as IL-2 and IL-5. This suggests that Th cell responses to self-antigens are strongly influenced by formation of ICs between the self-antigens and NAbs. Presumably, NAbs target self-antigens to APCs, as has been shown for polyclonal antibodies to foreign antigens, as well as for DAbs to the thyroid self-antigen, thyroid peroxidase (TPO).

Moreover, recruitment of T cells to the site of infection may also be influenced by NAbs and complement. Askenase and Tsuji demonstrated that T-cell-dependent contact sensitivity responses to hapten and subsequent rises in local IFN-γ levels were absent in pan B-cell- and antibody-deficient mice, but could be restored by adoptive transfer of purified normal peritoneal B-1 cells, or by i.v. injection of antigen-specific IgM monoclonal antibodies. They concluded that the contact sensitivity response was initiated by the formation of complement-activating ICs between hapten and naturally occurring IgM antibodies, followed by complement activation and C5a-mediated release of vasoactive substances by mast cells, facilitating the recruitment of T cells.

It remains to be clarified whether IC-formation with NAbs contributes to maintenance of tolerance, or whether it promotes breakage of tolerance. In mononuclear cell cultures from healthy individuals, grown in media containing autologous serum (30% v/v), TG induces immediate production of TNF-α and IL-10 by mononuclear cells, followed by an almost exclusive production of IL-10, a regulatory cytokine with a protective role in autoimmune diseases. A subset of T cells with a CD45RO memory phenotype seems to orchestrate this IL-10 production, suggesting that the TG-driven T-cell response in healthy individuals is protective and contributes to the maintenance of tolerance. By comparison, the foreign antigen tetanus toxoid induces no IL-10 production, but instead a mixed pro-inflammatory Th1/Th2-response (IL-2, IFN-γ, IL-4 and IL-5) under similar conditions. An absolute requirement for an intact tertiary structure of TG and MBP for induction of an IL-10 release by mononuclear cells supports a role for NAbs in maintaining tolerance.

DIFFERENCES BETWEEN AUTOANTIBODIES IN HEALTH AND DISEASE

NAbs and DAbs against cytokines and other self-antigens as discussed here, differ from one another in terms of isotype distribution and epitope recognition patterns. For example, in autoimmune thyroid disease most of the anti-TG activity is associated with IgG, with only ~1% in the form of IgM, whereas TG-reactive NAbs are predominantly of IgM isotype. Nevertheless, as much as 0.3% of IgG in IVIG preparations for intravenous use are reactive with TG or cytokines such as IL-1α and IL-6. There is evidence to suggest that the antibody recognition patterns of NAbs differ from those of DAbs. For example, the latter are more restricted in idiotypes and less polyreactive than NAbs. A certain idiotype, T44 Id, is associated with autoimmune thyroid disease and recognizes one of at least six epitopic clusters on human TG, designated region II. DAbs derived from sera of patients with Hashimoto’s thyreoiditis and Graves’ disease
recognized primarily region II and occasionally another region (region IV). By contrast, NAbS frequently react with region V and rarely with region II. Thus, recognition of region V may reflect the normal homeostatic recognition of TG.

Interestingly, the reactivity against particular epitopes commonly recognized by both NAbS and DAbs seems to change with aging, without affecting the total IgG anti-TG autoreactivity. We have recently demonstrated that NAbS to thyroid peroxidase (TPO) show a quantitatively different recognition pattern than DAbs from patients with HT. Anti-TPO NAbS recognize an immunodominant region involving two conformational, overlapping epitopes on TPO, referred to as immunodominant regions A (IDR-A) and B (IDR-B). In HT, approximately 50% of anti-TPO DAbs are directed to the IDR-B epitope, while DAbs against the IDR-A and non-A/non-B regions are approximately equally distributed. Interestingly, the propensity to produce autoantibodies directed against the IDR-A epitope of TPO seems to be inherited. We recently demonstrated that HT patients and their healthy, monozygotic co-twins had higher proportions of IDR-A-reactive anti-TPO antibodies (medians 19% and 18%, respectively) than healthy ordinary siblings to HT patients (9%) and euthyroid controls with no family history of HT (0%). These data confirmed the findings by Jaume et al. based on family studies that IDR-recognition patterns were genetically transmitted. In other words, the propensity to produce certain DAb reactivities may be inherited. Further studies are required to determine whether this applies to DAbs in general.

CONCLUSION

We have reviewed the immunoregulatory role of NAbS with special focus on autoantibodies against cytokines and other soluble self-antigens. Based on numerous publications, we and others believe that NAbS against cytokines and other self-molecules may in many cases contribute to homeostasis, and that DAbs may contribute to disease manifestations, in some instances perhaps as causative pathogenetic factors. These diseases likely include both autoimmune and autoinflammatory conditions. Moreover, TAbs may neutralize the effect of a number of “biologic” drugs and give rise to side-effects

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