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Chapter 8

Separation and Characterization of Leukocytes from the Intestine

P. B. ERNST,* A. D. BEFUS,† N. DYCK,* T. D. G. LEE,† AND J. BIENENSTOCK*

*Host Resistance Programme, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada L8N 3Z5 and †Department of Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada T2N 4N1

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I. Introduction

The mucosal surfaces of the body separate the external milieu from the internal environment and in so doing are exposed to stimulation from microbial and other antigens. These surfaces are protected, in part, by local immune responses that share common features which reflect the adaptation of the immune response to the mucosal environment (Bienenstock and Befus, 1980, 1985). For example, IgA is the predominant class of antibody in secretions whereas IgG makes up the largest proportion in serum. Furthermore, the migration pattern of mucosal immunoblasts differs from their systemic counterparts by having the propensity to accumulate selectively in mucosal tissues. The prevalence of IgA in secretions and the selective traffic of T and B immunoblasts among different mucosal tissues have led to the concept of a common mucosal immune system (McDermott and Bienenstock, 1979).

Some properties of mucosal responses differ from their systemic counterparts. This is illustrated by the observations that intestinal mast cells in the rat differ in a variety of ways from those seen in other sites such as the peritoneum (Bienenstock et al., 1985), and there is a predominance of
IgA-committed B cells and IgA-selective regulatory cells in mucosal tissues (Craig and Cebra, 1971; Tomasi, 1983) that help contribute to the high proportion of IgA in secretions. Thus, in order to clearly understand immunity in a mucosal tissue, one must utilize techniques to isolate cells from the tissue of interest and study them directly. This approach is preferable to developing models of mucosal immune reactivity by extrapolating from data obtained in studies of spleen or peripheral blood.

The intestinal mucosa is an excellent example of the unique conditions with which local immune responses must cope. The lumen contains a myriad of microbial and dietary antigens which are only separated from large populations of lymphocytes by the tight junctions of the absorptive epithelium. Furthermore, the epithelium is not an impenetrable barrier, so some antigenic molecules are constantly entering from the lumen. Thus, the interaction between antigen and T or B cells must be regulated to facilitate preventive responses to infection while limiting the magnitude of the reaction so epithelial integrity and function are not impaired by inflammation. Our lack of understanding of the immune responses in the intestine hampers our ability to control the toll taken by intestinal diseases in human or veterinary medicine. Therefore, it is important to seek more knowledge of local immune mechanisms by isolating cells from the intestine for direct study in order to enhance our ability to manipulate intestinal responses and help control disease in this tissue. This new information could be applied in the prevention of intestinal infection or the control of aggressive immune responses that may contribute to intestinal diseases such as celiac disease or inflammatory bowel disease (Ferguson, 1977; Ernst et al., 1986a).

The objectives of this chapter are to acquaint the reader with some of the questions and dogma that exist regarding intestinal immunity and to illustrate how the cells can be isolated and studied. The distribution and compartmentalization of cells in the intestine will be briefly reviewed before discussing their isolation. Methods will be described for the isolation of cells from the different compartments in the intestine of the mouse and, in addition, procedures for the isolation of cells from rat and human will be included. By including information on cell isolation from these different species, one readily appreciates the similarities or differences among species. The emphasis will be on what investigations have been done on cells isolated from the epithelium or lamina propria and how proper isolation is important to permit sound conclusions to be drawn from the data regarding the different populations. The data presented will demonstrate not only the difference between sites in the body, but also the compartmentalization that exists in the intestine itself.
II. Distribution of Lymphoid Cells within the Intestine

For studies of intestinal leukocytes, it is important to identify the compartment one wishes to study and then isolate a representative cross section of viable and functional cells. Subsequently, particular functional or phenotypic subsets can be obtained, studied, and incorporated into a model of local immunity in health or disease. Currently, it is believed that leukocytes are primarily compartmentalized in the intestine in either specialized sites for antigen penetration or other areas where various effector functions predominate. The compartments include the Peyer’s patches, the solitary lymphoid nodules, and the diffusely distributed leukocytes in the lamina propria or the intraepithelial region. These sites make up the gut-associated lymphoid tissue (GALT).

Antigen can be selectively taken up through the specialized epithelium (M cells) overlying the Peyer’s patches, where it then contacts antigen-presenting cells (Wolf and Bye, 1984). The Peyer’s patches have been shown to contain many regulatory cells including helper T cells, isotype-specific helper (Kiyono et al., 1984) or switch (Kawanishi et al., 1983) cells, suppressor cells (Richman et al., 1981a; Mattingly and Waksman, 1978), contrasuppressor cells (Green et al., 1982), and antisuppressor-cell circuits (Ernst et al., 1987). Thus, this tissue is of prime interest for studying early responses to enteric antigen and regulatory events.

In Peyer’s patches, precursors of cytotoxic T lymphocytes (CTL) (Kagnoff, 1978) and B cells, especially those producing IgA (Craig and Cebra, 1971), are present. Subsequent to the stimulation of T and B cells in Peyer’s patches, these cells are collected by draining lymphatics and pass to the mesenteric lymph nodes, thoracic duct lymph, and finally the blood. The blood then transports these blasts throughout the body and they are distributed throughout the entire intestine (reviewed by Bienenstock et al., 1983) and other mucosal tissues where they can mediate immune functions. The T and B cells repopulate different compartments in the intestine, as evidenced by the presence of T and B and plasma cells in the lamina propria while no B or plasma cells are found in the epithelium (Parrott et al., 1983; Ernst et al., 1985a, 1986b).

Other leukocytes are found in the lamina propria, epithelium, and/or Peyer’s patches, including mast cell precursors (Crapper and Schrader, 1983; Ernst et al., 1985b), polymorphonuclear cells (Tagliabue et al., 1982; Collan, 1972), veiled cells, and other antigen-presenting cells such as macrophages (Richman et al., 1981b; Frangakis et al., 1982; Kiyono et al., 1982; Spalding et al., 1983). At least some of these different leukocytes are located in more than one site in the GALT; however, their
distribution and functions have not been as well studied in the lamina propria or epithelium due to the relative difficulty in isolating cells from these two compartments. It is known that polymorphonuclear cells are generally not found in the epithelium of healthy intestine but reside in the lamina propria, while mast cell precursors can be found in high frequency in both compartments. The selective distribution of these and other cells has profound influence on the function of a particular compartment. New developments in cell isolation will allow studies of different cells in the lamina propria or epithelium to proceed and their role in host resistance to be assessed.

III. Isolation of Cells from Peyer’s Patches

The Peyer’s patches can easily be identified along the antimesenteric border of the small intestine of mice and rats; however, they are less frequently seen in humans. There are usually 6–12 patches visible in the small intestine of 4- to 6-week-old mice, and their size increases with age as they receive stimulation from luminal antigen. They can be removed by an elliptical incision and forced through stainless-steel screens into growth medium such as RPMI. This technique tends to provide preparations with only 70% viability, and it also almost totally destroys the non-specific esterase-positive cells and functional primary B cell responses in vitro (Frangakis et al., 1982). Thus, to increase yields of cells and enhance viability of preparations from Peyer’s patches, enzymatic digests have been used. One technique includes three to four (30 minutes) digestions of excised Peyer’s patches with Dispase (Boehringer Mannheim: Frangakis et al., 1982; Kiyono et al., 1982), whereas we have achieved excellent results (Ernst et al., 1987) with collagenase (Gibco) using a modification of a technique previously described by Richman et al. (1981b). The enzyme is added to growth medium, and warmed to 37°C; the Peyer’s patches are added to 30–50 ml of medium with the enzyme and then mixed at 37°C. The cell suspensions are pelleted and washed three times; they are then ready for use in an experiment. With either enzyme digestion >98% of the cells are viable, and they are fully competent to undergo a primary immunization in vitro to generate antigen-specific B-cell responses (Kiyono et al., 1982). Such preparations yield roughly equal percentages of T and B cells plus mononuclear cells that are responsible for antigen processing (Spalding et al., 1983). Peyer’s patch cells also have been cultured from immunized animals under conditions which select for T cells, and various helper T cell activities have been described in cells cultured in this fashion (Kawanishi et al., 1983; Kiyono et al., 1984).
IV. Isolation of Intraepithelial Leukocytes (IEL)

Leukocytes from the IEL make up approximately 10–15% of all cells in the epithelium and as such are a large population of cells that may contribute to protective or damaging responses that affect the epithelium. They are found on the luminal side of the basal lamina and, under appropriate conditions, separate with the epithelial cells from the underlying lamina propria. Although there have been many descriptions of techniques for isolating the diffusely distributed cells from the mucosa of mice, rats, guinea pigs, or rabbits (described by Ernst et al., 1985a), the most successful attempts have several factors in common as outlined below. These techniques allow one to study the morphology, chemistry, surface antigens, and functions of the isolated cells. With aseptic technique, the cells can also be grown for long periods of time in tissue culture.

When using mice, the technique described in detail by Tagliabue et al. (1982) and Davies and Parrott (1981a), with modifications (Petit et al., 1985), has been successful for our experiments and is applicable to different strains of mice. Following the removal of the intestine from 5–10 mice, luminal contents are flushed out; Peyer’s patches are removed, and the gut is opened lengthwise. Pieces measuring 2–3 cm long are then incubated in Ca- and Mg-free Hanks’ basal salt solution (HBSS) with 10⁻⁴ M EDTA (Tagliabue et al., 1982). With this procedure, the epithelium and IEL can be separated from the lamina propria by two or three (15 minutes) incubations at 37°C with gentle mixing. This yields a mixture of viable IEL, viable epithelial cells, and dead cells (almost exclusively dead epithelium). Repeated washing is performed before rapid filtration through nylon wool columns to remove clumps of dead cells. Cells are washed again in HBSS and then in 30% Percoll (50 ml for five mice: Petit et al., 1985). This step removes a great deal of mucus and dead cells, which can impair the yields and purity obtained in subsequent steps. The pellet from this is put onto a 50-ml discontinuous Percoll gradient, centrifuged, and cells harvested from the 55% (IEL55) and 70% (IEL70) Percoll fraction. These fractions include 50–55% of the cells put on the gradient, and 85–95% of these are leukocytes—at least 90% of which are derived from the epithelium versus the lamina propria (Ernst et al., 1986b).

One factor that dramatically affects the yield and purity of IEL preparations is the level of antigenic stimulation experienced by the mice. Specific pathogen-free mice have fewer microbial challenges, and this is reflected with fewer T cells in IEL (Carman et al., 1986). We have also observed that the yield of lymphocytes from the Peyer’s patches and lamina propria of these mice is also lower, which suggests that very clean
housing conditions lead to less stimulation, lower T and B cell numbers, and less purity in the cell preparations.

Enrichment with cell elutriation, the addition of a multistep gradient, or separation by velocity sedimentation is required to consistently accomplish complete removal of epithelial cells. This extra effort is sometimes warranted, since their presence has been shown to inhibit functional activities such as natural killer (NK) activity (Targan et al., 1983). We have been able to obtain adequate purity of the mouse intestinal leukocytes to see NK activity with the discontinuous Percoll gradient (Tagliabue et al., 1982), which is less time-consuming than the other two techniques.

Another technique which may be more rapid and can yield preparations with adequate purity has been developed in the rat using a modification of epithelial cell isolation described by Bjerknes and Cheng (1981). Briefly, an intravenous infusion of EDTA followed by vibration of the inverted gut causes the epithelium to float off the mucosa. Subsequently, using nylon wool, repeated washings, and gradients (Mayrhofer and Whately, 1983; Huntley et al., 1984), the IEL can be separated. With techniques similar to those described above, IEL separation can be done without excessively high concentration of EDTA, enzymes, or undue trauma, which may liberate more cells from the lamina propria (thereby contaminating the IEL preparation) or result in a decrease in functional activity (Chiba et al., 1981; Gibson et al., 1984). This is a critical consideration for planning investigations into the function of cells from the epithelium which are to be compared to cells from the lamina propria.

V. Isolation of Cells from the Lamina Propria

For the isolation of leukocytes from the lamina propria (LPL) of mouse intestine, the IEL must first be removed as described above. Multiple washing of intestinal segments with HBSS and EDTA (Davies and Parrott, 1981a; Carman et al., 1986) eventually produces clear supernatants, suggesting that all epithelial cells and IEL have been removed. The gut is then cut into small pieces before being incubated twice (45 minutes) at 37°C with collagenase. The effectiveness of different brands and lots of collagenase differs considerably, so each one must be tested to determine an effective concentration at which to digest leukocytes out of the lamina propria (Tagliabue et al., 1982; Davies and Parrott, 1981a). Following digestion of the intestinal tissue, the cell suspensions are processed in essentially the same fashion as described for IEL (Tagliabue et al., 1982). They are rapidly filtered through gauze to remove tissue debris, washed repeatedly in HBSS, and purified over a discontinuous Percoll gradient.
The LPL differ in a number of ways from IEL and can be determined to be relatively free of IEL if the unique granulated IEL are absent. The cells from the different compartments in GALT are compared in more detail below.

As stated earlier, in order to assess the role of a particular mucosal cell population in a disease process, the characteristics of that population need to be established directly rather than from extrapolations from systemic cell populations. Mast cells from the intestinal tract have attracted much attention because of their potential role in inflammatory bowel disease, celiac disease, food allergy, and antiparasite immunity (Dvorak, 1979; Heatley et al., 1979; Strobel et al., 1983; Saavedra-Delgado and Metcalfe, 1983; Befus and Bienenstock, 1982). Rat intestinal mast cells (IMC) were shown to be histochemically different from peritoneal mast cells (PMC), and subsequently both PMC and IMC have been compared morphologically and functionally (Befus et al., 1982). These studies have also been initiated with cells from human and nonhuman primates (Fox et al., 1985; Barrett and Metcalfe, 1985).

To isolate and purify these cells to an extent that they could be studied directly, we began with rats infected with the nematode Nippostrongylus brasiliensis (Nb), at a time postinfection (35 days) when there was still mast cell hyperplasia in the intestine but when the gross inflammation and villous atrophy initiated by this worm had diminished. The technique is described in detail by Lee et al. (1985) but is summarized as follows. The entire rat small intestine is removed and quickly flushed with saline at room temperature. After removal of the Peyer's patches and mesentery, the pieces of intestine are washed well in HBSS and then cut into small (1–2 mm) pieces to be incubated at 37°C in three (10 minutes) changes of HBSS with EDTA. Between each EDTA incubation, the tissue is washed quickly in HBSS to reduce mucus and debris. This is followed by two (1 hour) incubations at 37°C with collagenase (Gibco). Supernatants are collected and filtered through gauze. The cells from the first collagenase incubation are discarded because of the presence of large numbers of eosinophils. After removal of cells from the supernatant of the second incubation by centrifugation, the remaining tissue is mechanically disrupted by repeated syringing (~20 times with a 10-ml syringe in a 100-ml beaker) in RPMI 1640. Cells recovered from the second collagenase incubation and from syringing the remaining tissue are pooled, washed, and cleared of coarse debris by rapid passage through a nylon wool column into a 50-ml polystyrene tube (Corning, New York, New York—glass is to be avoided as the mast cells will stick to glass). The resulting cell suspension is centrifuged (200 g, 10 minutes, 15°C) and resuspended very gently using a siliconized-glass pipet in supplemented RPMI (as above).
Cell suspensions are enriched for IMC by layering not more than $1 \times 10^8$ total mucosal cells (viable plus nonviable) in 10 ml on discontinuous density gradients of Percoll. Gradients are formed in clear 50-ml polystyrene tubes (as above) and consist of a bottom layer of 15 ml of 80% Percoll and an upper layer of 20 ml 30% Percoll. (This gradient is stable for up to 1 hour at room temperature.) Following centrifugation, the cell pellets are resuspended very gently with a plastic or siliconized-glass pipet in a small volume of supplemented RPMI 1640. Further enrichment of IMC is achieved based on cell size using velocity sedimentation at unit gravity.

This technique of isolating pure mast cells from the intestine is invaluable in the study of gastrointestinal disease in that it provides a population of cells which can be carefully analyzed for their response characteristics, mediators secreted, and response to pharmacological modulation. Of particular interest are the characteristics of the response to mediators secreted by other lamina propria cells (such as lymphocytes) and their abilities to influence the behavior of other cells by their mediator secretion.

Table I shows the composition of the cells obtained at three different stages of the isolation and purification procedure. Lymphocytes are the main contaminating population at all stages, representing nearly 30% of the total viable cells in the initial isolated lamina propria population. Mast cell purity rises from 35% in the initial population, to 65% in the Percoll-enriched population, to >95% in the population purified by sedimentation.

| Cell type         | Initial isolation $(n = 8)$ | Percoll-enriched $(n = 8)$ | Fraction Sta-Put $^b$ $(n = 4)$ | Total viable cells (%) |
|-------------------|----------------------------|---------------------------|---------------------------------|------------------------|
| Mast cells        | 35.2 ± 3.2                 | 65.5 ± 5.2                | 95.7 ± 1.3                      | (±SE)$^a$              |
| Epithelial cells  | 7.4 ± 3.6                  | 0.9 ± 0.3                 | 0.3 ± 0.3                       |                        |
| Lymphocytes       | 29.4 ± 7.9                 | 15.3 ± 3.2                | 3.5 ± 1.6                       |                        |
| Eosinophils       | 11.7 ± 3.2                 | 3.6 ± 3.7                 | 0.5 ± 0.4                       |                        |
| Other cells$^c$   | 16.0 ± 1.6                 | 14.7 ± 3.7                | 0 ± 0                           |                        |

$^a$ Modified from Lee et al. (1986).

$^b$ Purified by velocity sedimentation using Sta-Put apparatus.

$^c$ Other cells include macrophages, polymorphonuclear cells, etc.
velocity. Our studies have shown that the mast cells in all three preparations are similar in their size, histamine content, protease II content, and responses to release agents, which makes us confident the different preparations are representative of the entire IMC population.

The application of cell isolation techniques to human intestine permits investigations to be correlated to clinical conditions. The size of the human bowel and the presence of a profuse mucous layer requires special consideration. Fortunately, most of the difficulties encountered with these physical features have been overcome. IEL have been isolated and studied by Cerf-Bensussan and colleagues (1985), and their preparations yielded highly purified IEL populations. Most of the other reports in the literature describe techniques for the isolation of the LPL or mixtures of LPL and IEL, which have led to new information about intestinal immune responses (Bull and Bookman, 1977; Fiocchi et al., 1979; Chiba et al., 1981; Gibson et al., 1984). This work has been the topic of a review by Pena (1985). Mast cells can also be isolated from human intestine, which can facilitate studies of human mast cell heterogeneity and responsiveness to antiallergic compounds. One technique has been described by Fox et al. (1985), whereas the technique we have used for isolation of mast cells from the lamina propria of human intestine is as follows (Befus et al., 1987).

The mucosal layer is removed in strips of about 0.5 × 0.3 cm from the muscle and adherent connective tissue, rinsed in HBSS, cleaned of surface debris by blotting with bibulous paper, and weighed. Typically 3–7 g (wet weight) of mucosa is obtained from each specimen. To remove mucus and minimize epithelial cell contamination, the tissue is incubated for two 90-minute periods at 37°C with gentle stirring in 100 ml of Ca- and Mg-free buffered HBSS containing 1 mM EDTA and antibiotics. Following the first 90-minute incubation, the suspension is filtered through cheesecloth; the mucosal strips are then rinsed thoroughly in HBSS and added to fresh EDTA solution for the second 90-minute incubation, after which the tissue is again rinsed in HBSS. The mucosa is cut into small pieces (2 mm³) and held overnight (16 hours) at 4°C in 100 ml of HBSS with antibiotics.

Collagenase is added the following morning and the tissue is incubated at 37°C with gentle mixing for 2–3 hours. Following digestion with collagenase, the suspension is filtered and washed in polystyrene tubes at 200 g for 8 minutes at room temperature and resuspended in HBSS. Repeated washings yield a sample of human intestinal leukocytes (10 × 10⁶ leukocytes per gram of mucosa, 95% viable, 8% mast cells) that can be studied directly or after further enrichment using density gradients, velocity sedimentation, or elutriation.
VI. Problems Associated with Isolation

The selection and development of a good isolation protocol is essential, since the functional capability of the isolated cells may depend on the viability following isolation. Excessive trauma or chemicals used in the isolation may also impair functions such as NK activity (Chiba et al., 1981; Gibson et al., 1984). Also, overly aggressive isolation of IEL may result in large numbers of LPL entering the preparation, thereby giving misleading results when one is interested in attributing a particular function to one or more compartments. This is illustrated by the disagreement in the literature regarding the number of IEL related to B cells. In situ staining of human bowel has failed to show up immunoglobulin-positive cells in some reports (Selby et al., 1981), which is in disagreement with others (Bartnik et al., 1980). Thus, the description of immunoglobulin-positive isolated IEL is difficult to interpret, since they actually may be a result of contamination by LPL, which are rich in such cells. Descriptions in mouse (Carman et al., 1986; Parrott et al., 1983) and rat small intestine (Lyscom and Breuton, 1982) clearly suggest that IEL are free of B cells and, in the opinion of the authors, this is likely the situation in humans as well.

Inadequate separation of IEL from enterocytes also affects the function of cells. For example, the presence of NK activity in IEL has been controversial; however, by separating enterocytes using multistep Percoll gradients (Tagliabue et al., 1982), cell elutriation (Targan et al., 1983), or sedimentation velocity (Ernst et al., 1986b), NK activity can routinely be demonstrated. In fact, epithelial cells have been shown to inhibit systemic NK activity (F. Shanahan and S. Targan, personal communication). Therefore, ideal cell fractionation requires good resolution but must also provide a representative sample.

The observation of IEL or LPL lacking a particular phenotype or function cannot be interpreted easily, since the negative result may be due to the isolation procedure. This can be tested by putting a positive control cell population through the isolation procedure. This only approximates the situation which occurs with IEL or LPL isolation, since epithelial cell contents, enzymes, and other mediators from the tissues may be responsible for negative data. A better experiment entails the mixing of cells expressing the trait (positive control) with gut segments, performing the isolation procedure and comparing the expression of the trait by the mixture of isolated positive control cells and mucosal leukocytes. Presumably, the properties of the positive cells will be preserved and express some degree of activity and thereby verify that the isolation procedure is not responsible for negative results. If this type of control (albeit a tedious
one) is performed, one would have more confidence when interpreting negative data. An example of this investigation into the effect of the isolation procedure on mast cell secretion is illustrated in Table II. In this example, IMC had little response to the potent PMC secretagogue 48/80. However, to show that the isolation procedure did not effect this response, PMC were isolated in the normal fashion and compared with PMC put through the protocol used for IMC isolation. These data show that the isolation procedure did not markedly interfere with mast cell function. This makes the conclusion that IMC do not react to 48/80 more valid.

VII. Properties of Intestinal Leukocytes

A. Morphology

The compartmentalization of the cells in the GALT is immediately evident on morphological criteria alone. The IEL distinguish themselves by being almost entirely mononuclear cells resembling lymphocytes (Collan, 1972), while the lamina propria contains numerous polymorphonuclear cells. The IEL are also unique in that a large percentage contain azurophilic cytoplasmic granules. Such granulated cells have been described in the epithelium of mice, rats, rabbits, guinea pigs, chickens, and humans in both the small and large intestine (reviewed by Ernst et al.,
1985a). In our studies using CBA/J mice, approximately 55–65% of the IEL were shown to contain granules. This unusual morphological subset is rarely seen in the lamina propria, and its enrichment in the epithelium suggests they carry out a function that is adapted to this compartment.

The presence of cells resembling macrophages in the lamina propria and Peyer’s patch suggests these compartments can process antigen and initiate an immune response. Consistent with this are the observations that the Peyer’s patches are fully competent at generating a B cell response to antigen in vitro (Kiyono et al., 1982).

Polymorphonuclear cells and mast cells increase their numbers in inflammatory responses occurring in the lamina propria. The mast cell hyperplasia can also extend to the IEL compartment, especially in association with parasitic infestations with *Trichuris* or *Trichinella* sp. Thus, the distribution of different morphological subsets probably reflects specific functions of a compartment in the overall immune/inflammatory response.

### B. Surface Antigen Phenotype

The compartmentalization and heterogeneity of mucosal cells is more evident by their surface antigen phenotype. The IEL again distinguish themselves in that a high percentage (80–85%) express the Lyt-2 antigen in the mouse even though only 30–35% are Thy-1+ (Parrott et al., 1983; Petit et al., 1985). Thus, approximately 50% are Thy-1−, Lyt-2+ and have been shown to be granulated and, thus, represent the majority of the granulated IEL. Cells with this phenotype have not been described in the lamina propria or Peyer’s patch. Similarly, granulated IEL from humans and rats express OKT8 or OX8 antigens, respectively, while lacking the pan T cell marker Leu-1 or W3/13. To date, these cells have not been shown to have a relationship to mast cells, NK cells, or cytotoxic T lymphocytes, despite specific attempts to make these associations. The lineage and function of this subset remains obscure; however, it is possible to isolate this cell and study this subset directly in order to determine their function.

Cells with surface or cytoplasmic immunoglobulin are also restricted in their distribution. Such cells can be found in Peyer’s patches and lamina propria; however, they are almost totally lacking in IEL of mice and rats. As mentioned earlier, there are conflicting reports about their presence in human IEL preparations; however, based on our observations and those of Selby et al., (1981), they are probably absent in humans as well.

The number of cells expressing Ia antigens have also been shown to vary significantly from one compartment to another in GALT and also...
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TABLE III

COMPARISON OF THE SURFACE ANTIGEN PHENOTYPE OF CELLS FROM PEYER'S PATCHES, LAMINA PROPRIA, AND EPITHELIUM OF THE SMALL INTESTINE OF THE MOUSE

|       | Surface | Thy-1 | Lyt-1 | Lyt-2 | AsGM1 | Ly-5 | Ia  |
|-------|---------|-------|-------|-------|-------|------|-----|
|       | Ig      |       |       |       |       |      |     |
| Peyer's patch | 40–50 | 30–40 | 30–40 | 5–10  | ?     | 90   | 50–60|
| LPL    | 30–40 | 40–60 | 40–60 | 30    | 10    | ?    | 10–15|
| IEL    | <5     | 30–40 | 30–40 | 70–80 | 15    | 80–90| <1  |

Data reflect a range of values that have been reported in the literature by different laboratories (see text).

have been shown to change with inflammation. They are expressed on B cells, antigen-presenting cells, and some T cells, so it is not surprising to find them in the highest amounts in the Peyer's patches while IEL apparently lack this marker. Inflammatory processes are also associated with the expression of Ia antigens by the epithelium itself. Whether the enterocytes that acquire this trait may then be able to participate as antigen-presenting cells is unknown.

Table III shows the proportion of cells expressing various markers in the different compartments of GALT. The values that are shown reflect our interpretation of many observations from other investigators (Richman et al., 1981b; Parrott et al., 1983; Guy-Grand et al., 1984; Dillon and MacDonald, 1984; Alberti et al., 1985; Carlson et al., 1986), in addition to our own. The values vary from one study to the next depending on the isolation procedure, reagents, strain of mouse, etc. The key feature is the selective distribution of some subsets and the merit in comparing different compartments. This phenotypic heterogeneity clearly is mirrored by functional heterogeneity and emphasizes the specialized role of each compartment. Thus, new functions for cells in GALT should be compared with other compartments and tissues to assess their respective roles in host resistance.

C. FUNCTIONS

1. Antigen Presentation and Immune Regulation

The functional capabilities of the different compartments within GALT have also been studied in some detail. The Peyer's patches have been incriminated as the preferred site of antigen uptake from the lumen and a key location for the generation of an immune response. This is facilitated by the specialized epithelium (M cells) overlying the patch, which can
take up antigen more easily and is often the preferred site of attachment of microbial agents.

Having penetrated the Peyer's patches, the antigen may encounter antigen-presenting cells and a host of regulatory T cells which are required for initiating an immune response (Bienenstock and Befus, 1985). Regulatory T cells have been described by various investigators and include T-helper cells, T-switch cells, IgA-specific helper cells, contrasuppressor circuits, and the antisuppressor pathway (see above). Our understanding of events in this site is relatively great due to the ease with which these cells can be isolated. This is not the case for the diffusely distributed cells in the epithelium or lamina propria. The role of cells in these sites (IEL, LPL) in generating a response is largely unknown, even though antigen can pass through absorptive epithelium and contact IEL and LPL directly. The ability of those compartments to react to antigen may be limited if they lack some of the necessary cells to generate a response. For example, IEL have not yet been shown to possess antigen-presenting cells or some T cell regulatory circuits (i.e., antisuppressor pathway); therefore, they may not be capable of generating a response. Despite this absence of cells needed to generate a response, IEL do contain cytotoxic T-lymphocyte precursors. It is possible that the adjacent epithelium or LPL may provide the necessary assistance to initiate a response by precursors in this site.

2. Cytotoxic Cells

The presence of CTL and/or CTL precursors (CTLp) has been detected in Peyer's patches, lamina propria, and the IEL. For example, Kagnoff (1978) has shown that following oral immunization with allogeneic tumor cells, CTL can be detected in Peyer's patches. Intraperitoneal injection, with or without oral feeding, can also induce alloantigen-specific CTL in IEL and LPL (Klein and Kagnoff, 1984; Davies and Parrott, 1980,1981b). Precursors for these cells may have been in the compartment initially or may have arrived by migration from another site following immunization. IEL and LPL have been isolated and shown to contain CTLp (Ernst et al., 1986b), which suggests that these cells can be stimulated in situ by lumenal or epithelial associated antigen.

GALT also contains various naturally cytotoxic or NK cells. IEL, LPL, or combinations of both have been shown to have NK activity in mice, rats, guinea pigs, and humans (reviewed by Ernst et al., 1985a). IEL may also be easily activated by regulatory molecules (interleukin 2, interferon) to give cytotoxic cells with a broad pattern of specificity. This has been observed by Ernst et al. (1985b) and Klein et al. (1985), who have demonstrated that IEL can be cultured and activated to give killer cells with a broad specificity. Furthermore, oral and peritoneal immuniza-
tion has also been shown to give rise to "activated killers" that are not usually seen in spleen (Klein and Kagnoff, 1984). IEL from mice have also been shown to contain a cell with NK activity against targets infected with an enteric virus (Carman et al., 1986). This antiviral activity mediated by this cell in the epithelium is 4–10 times greater than the activity in the Peyer's patch, mesenteric lymph node, or lamina propria, which again shows the compartmentalization of effector cells in GALT. The IEL, as mentioned above, have a large population of granulated cells which resemble the large granulated lymphocytes which mediate NK activity. However, our data have shown that the majority of these cells in mice are not associated with the killing of YAC-1 or virus-infected targets.

3. Mast Cells

As discussed earlier, the GALT also has a propensity to undergo mast cell hyperplasia in the presence of chronic inflammation, especially in association with an infestation of parasites. The mast cells can be seen in the lamina propria and the epithelium. Interestingly, during infestation with Nb most of the hyperplasia occurs in the lamina propria, while dramatically more mast cells are seen in the epithelium in association with infestations of Trichurus or Trichinella sp. Until recently, there was little information to clarify whether the intestinal mast cell hyperplasia was a result of local precursors differentiating or an infusion of cells from the blood. With mucosal cells from the mouse intestine, it has been shown that IEL and LPL contain an extraordinarily high number of mast cell precursors. There are approximately 10 times more in gut than bone marrow, 100 times more than spleen, and 100 times more than peripheral lymph nodes (Crapper and Schrader, 1983; Guy-Grand et al., 1984). This would suggest that, during inflammation, IMC are derived principally from local precursors. Such high numbers of precursors also suggest they play a key role in protecting the intestine; however, the exact nature of their functions is unknown.

The mast cells present in the intestine of rats during infection with Nb have been isolated as described earlier. These, in turn, have been compared to mast cells from the peritoneal cavity and lung, and have helped develop the concept of mast cell heterogeneity. The heterogeneity of mast cells from different sites is obvious morphologically and is substantiated further based on staining characteristics, biochemical properties, and responses to secretagogues or antiallergic compounds. The reasons for this heterogeneity (lineage, microenvironment) remain to be clarified in more detail, although some information suggests that the microenvironment is important (Nakano et al., 1985).

Mast cell heterogeneity is expressed among species, among individuals
TABLE IV
MAST CELL HETEROGENEITY IN THE RAT: FACTORS THAT INDUCE MEDIATOR SECRETION

| Factor                                      | Peritoneum | Skin | Lung | Intestinal mucosa |
|---------------------------------------------|------------|------|------|-------------------|
| Antigen, anti-IgE                           | ++         | +    | ?    | ++                |
| 48/80, Bee venom peptide 401                | ++         | +/0  | +    | 0                 |
| T-Cell factor                               | ++         | ?    | ?    | ?                 |
| Ionophores                                  | ++         | +    | +    | ++/++             |
| Substance P                                 | ++         | ?    | +    | +                 |
| VIP, somatostatin, bradykinin, neurotensin  | ++         | ?    | ?    | 0                 |
| Dynorphin, β-endorphin, α-neoendorphin      | ++         | ?    | ?    | 0                 |

(or strains) within a species, among different sites in an individual, and among cells at a specific site. Table IV, for example, illustrates the major differences seen in mediator secretion characteristics between mast cells isolated from the rat peritoneal cavity, skin, lungs, or intestinal lamina propria. In addition to these differences in responsiveness to release stimuli, the panel of mediators secreted by mast cells shows considerable variation. Rat PMC and IMC, for example, secrete different stored mediators such as the chymotryptic proteases rat mast cell protease I and II (RMCP-I, PMC; RMCP-II, IMC: Woodbury et al., 1978), and the evidence is suggestive that they also secrete different newly formed mediators such as the arachidonic acid metabolites (leukotrienes and prostaglandins) upon stimulation (Razin et al., 1982, 1984; Mencia-Huerta et al., 1983). Such mediator differences suggest that mast cell influence at all sites is not identical and that the responses orchestrated by mast cell products could be substantially dissimilar.

This heterogeneity is of considerable importance to our understanding of the role of mast cells in health and disease. Characterization of form and function of a particular mast cell population isolated from a particular site cannot be used to extrapolate directly to form and function of other mast cell populations at the same or at other sites. Heterogeneity is therefore an important consideration when assessing the role mast cells play in disease processes. Moreover, mast cell heterogeneity has added clinical significance as a consideration for relevant therapeutic intervention.
This chapter has attempted to expose the reader to the concept that intestinal immune mechanisms must be studied directly and incorporated into models based on observations made using intestinal cells and not just systemic cells. Numerous reports in the literature compare and contrast mucosal and systemic responses; however, despite their tissue-specific variations, the different tissues do interact. Compartmentalization of the immune response is substantiated further by the fact that within the GALT, each compartment (Peyer's patch, mesenteric lymph node, lamina propria, and epithelium) possesses its own characteristic and heterogeneous population of cells. This obviously has broad implications regarding the predominant functions or roles of the respective compartments in the overall immune response.

Fortunately, sufficient methodologies exist which allow each compartment to be separated free of each other. This is critical if one is interested in attributing a function to a particular compartment. Sufficient data exist in the literature to allow one to phenotype the cell preparation from each compartment and estimate their purity and allow subsequent comparisons of various subsets. Relating the data from isolated cells to phenotypes of cells described in the literature from in vitro and in situ studies will allow one to estimate the yield of a cell preparation and determine whether it is representative of a particular compartment. Using this approach, one may further define or modify the descriptions of cells in a particular compartment as well as determine the function of a particular location.

The procedures used to isolate cells from GALT often are traumatic or include enzyme digestion. A technique should be selected that is efficient yet gentle enough to provide viable and functional cells. Negative results must be interpreted with caution, since the isolation procedure may inhibit function or alter the phenotype. Similarly, cells contaminating leukocyte preparations can affect functional assays. This necessitates that positive controls are put through the procedure or even added to intestinal pieces before isolation. If these cells demonstrate a positive response following this handling, then one is more confident of negative results observed in isolated intestinal cells.

In conclusion, the selection and verification of an isolation technique is dependent on several variables. The current level of understanding of GALT is sufficient that researchers may design experiments which can be well controlled and used to ascertain the contribution of different cells and compartments within GALT to the prevention and pathogenesis of enteric disease.
ACKNOWLEDGMENTS

The authors acknowledge the support of the Medical Research Council of Canada, the Canadian Foundation for Ileitis and Colitis, the Alberta Heritage Foundation for Medical Research, and the Rockefeller Foundation. The secretarial services of Janice Butera and Barb Lahie were greatly appreciated.

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