The Basis for the Immunoregulatory Role of Macrophages and Other Accessory Cells

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Macrophages handle extracellular proteins and secrete diverse bioactive molecules and, therefore, influence the physiology of many tissues. They also have an important immunoregulatory role. The immune response to proteins involves the activation of the T helper subset of lymphocytes. The T helper cell is activated only when it interacts with the protein displayed on the surface of a macrophage or other accessory cell. This interaction involves restrictive proteins encoded in the major histocompatibility gene complex as well as growth-differentiating proteins.

THE MONONUCLEAR PHAGOCYTE SYSTEM, CONSISTING OF monocytes and tissue macrophages, is the major cellular component of the classical reticuloendothelial system. It is a dynamic cellular system with representation in all tissues and with the potential to exert a modulatory role in tissue homeostasis and in local immunological and inflammatory responses. The initial concept—stemming from the early experiments of Metchnikoff—that the macrophage is a scavenger of the extracellular environment in search of debris and unwanted material is an underestimate of the powerful role of macrophages. These phagocytes are highly active cells that readily respond to hormonal and cellular signals and therefore participate in a variety of physiological and pathological events.

Macrophages have a unique place in the tissue response to external stimuli (*1*). First, they can interact with many extracellular molecules—proteins and polysaccharides—and can internalize and submit them to intracellular metabolic changes. These molecules may be free in solution or form part of the structure of microbes. Second, macrophages are highly secretory cells. The secretory products include proteases, complement proteins, growth regulatory factors such as interleukin-1 (IL-1), and arachidonate derivatives. All of these molecules are important in inflammatory reactions. The secretion of many of them depends on the metabolic state of the macrophage, which in turn depends upon the interaction between the macrophage and its surroundings. Third, macrophages interact with the T and B lymphocytes and thereby intervene in immunological responses. Fourth, macrophages are critically situated in the various tissues, usually close to the microvasculature and surrounding epithelial and mesenchymal cells. Fifth, macrophages have surface receptors for lymphokines, the regulatory proteins released by lymphocytes; upon interaction with lymphokines, macrophages acquire novel properties included under the term "activation." Activated macrophages are highly microbicidal and tumoricidal. Thus, the mononuclear phagocyte system is involved in infectious processes, in the modulation of immunological responses, and in inflammation.

The many roles of macrophages can best be appreciated by the analysis of immunological reactions. In these reactions, the involvement of macrophages begins with the early events that lead to stimulation of lymphocytes and induction of a response and extends to the effector inflammatory reactions that characterize cellular immunity (known by the term delayed hypersensitivity). In this article, we review the biology of the macrophages, mainly in the context of immunological interactions, in which the function of macrophages has best been studied. General principles applicable to other cellular interactions can be derived from the immunological studies.

The interactions between macrophages and lymphocytes are noteworthy in showing the extent of interdependency of both cells. We emphasize two important points. (i) In the process of uptake of proteins by the macrophage, part of the protein molecule is salvaged from extensive proteolysis and becomes accessible to the immune system in a form compatible for immunological recognition. Many of the protein antigens are subjected to a biochemical processing event that changes their structure, so that what is recognized by the cellular immune system is distinct from the native protein. The scavenger concept, therefore, has to be profoundly modified. Indeed, studies involving macrophages were the first to reveal these changes in protein antigens. (ii) Macrophages and lymphocytes modify the behavior of each other—in part, through the release of bioactive molecules such as interferon-γ (γ-IFN) and IL-1. The interactions between macrophages and lymphocytes and the release of these products are under critical control.

Inductive Immunological Reactions

The cellular events that result in the establishment of an effective immune response are highly complex. This should be expected from a multicellular system that needs to (i) have recognition structures for a diversity of foreign molecules, (ii) discriminate between foreign and related autologous molecules, and (iii) place rapidly into operation multiple cellular effector systems. The central step in the development of responses to proteins and peptides is the activation of helper T cells (to cellular immunologists, activation means both the growth of antigen-specific clones of lymphocytes and their differentiation to an effector function; the latter is usually manifested as secretion of bioactive molecules or the development of a specific response, such as cytotoxicity or phagocytosis) (*2*). The helper T cell is the subset distinguished by expression of the CD4 molecule and

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by secretion of a number of modulatory molecules. Helper T cells are not activated by direct interaction with foreign proteins and are required to recognize them on the surface of cells. Accessory cells that take up the foreign proteins and serve as the substrate for recognition by the CD4-containing T cell are now included in the term "antigen-presenting cells" (APC).

Definition of accessory cell function. The history of our understanding of accessory cell function has been reviewed (*3*). In brief, experiments in the early 1960s indicated that uptake of proteins by the reticuloendothelial system correlated well with the immunogenicity of a protein. Transfusion of live macrophages containing a radioisotope-labeled foreign protein to unimmunized mice resulted in strong stimulation of an immune response. With some proteins, one of us (E.R.U.) with B. A. Askonas, showed that the macrophage-associated antigen was 1000 times as potent an immunogen as antigen alone, even though 90 percent of the molecules taken up by the macrophages were entirely degraded. Subsequent approaches (*3*), first reported by Mosier (*4*), indicated that lymphocytes in culture would respond poorly, if at all, if accessory cells were depleted by their differential adherence to culture surfaces. A breakthrough in the understanding of accessory cells came from the studies of Rosenthal and Shevach in 1973 (*5*), which indicated that the major histocompatibility gene complex (MHC) was involved in the interaction between macrophages and T cells. The MHC codes for two families of cell surface glycoproteins termed class I and II (*6*). Class I molecules are composed of two chains, a heavy chain of approximately 44 kilodaltons and a small associated peptide, β_2-microglobulin, of approximately 15 kD. Class I molecules are present on the surfaces of all cells and include the classical transplantation antigens. Class II molecules (or Ia molecules—we use the two terms interchangeably) are heterodimers made of 34- and 28-kD chains found mainly on the surfaces of macrophages, B cells, and the Langerhans-dendritic cells of the skin and lymphoid organs. Early studies indicated that a T cell interacting with a protein presented by an autologous macrophage would not respond to the same protein presented by a macrophage bearing a different class II allele. (The MHC is highly polymorphic, with each locus having 20 to 50 alleles; in each species several loci encode different MHC class I or II molecules.) The MHC imposed a "restriction" on recognition. At the same time that MHC restriction was found for macrophage–T cell interaction, a similar phenomenon was found for the interactions of B cells with T cells and for cytolytic T cells with their targets (*7*). Cytolytic T cells, which express the CD8 protein, represent the second stable subset of T cells.

The studies on antigen presentation brought together two basic immunological observations. The first concerned the biology of transplantation. The MHC had been discovered in the course of transplantation reactions, but the physiological significance of these diverse surface antigens had baffled immunobiologists for years. The antigen presentation studies provided the first examples of these proteins playing a role in normal cellular interactions. The second dealt with the immune response genes (Ir genes) discovered by the laboratories of McDevitt and Benacerraf (*8*). They found that responses to synthetic peptides of relatively simple structure—that is, random copolymers made of one to four amino acids—varied among inbred strains of animals. Some responded strongly, others very weakly. These differences were best shown with inbred strains of mice that differed in all or part of the MHC (called H-2 in the mouse). The trait of responsiveness was mapped to H-2 and to a region termed I, later found to code for the MHC class II or Ia molecules. Subsequent studies made it clear that the MHC class II molecules expressed on the APC system were the key molecules that regulated the capacity to make a cellular response directed against protein.

Cellular studies of the past 10 years have given us insights into the basic principles of the T cell recognition system accepted by most immunologists. (i) CD4-positive T cells recognize proteins only when the proteins are presented to the T cells by an APC that bears an MHC class II molecule. Since MHC class II molecules are expressed on only a few cells, this limits immune recognition and bars recognition of proteins, including autologous ones, on cell types that do not bear an MHC class II molecule—that is, on most of the cells of the body. (ii) The allele of MHC class II is one factor that establishes which antigenic determinant is recognized. Some sequences are recognized only by a given allele. In the case of the natural proteins, which have several antigenic determinants, "responders" or "nonresponders"—such as described for the simple polymers referred to in early studies—are seldom found. However, the immunodominant regions or epitopes of the protein vary depending upon the MHC class II allele involved in its presentation. (iii) The activation of the CD4-positive cells initiates the diverse cellular interactions that result in B cell activation, development of

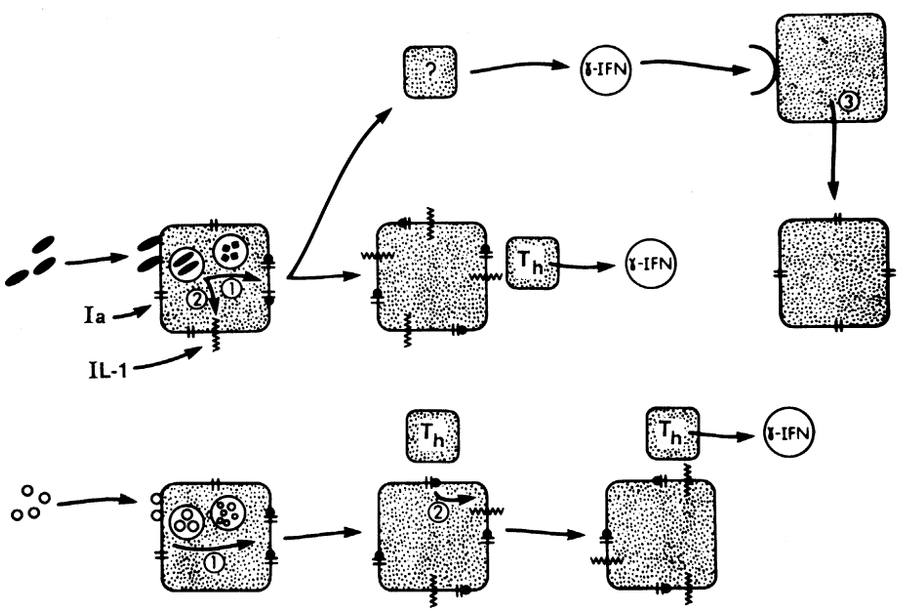


Fig. 1. The events involved in antigen presentation by macrophages. Most proteins require internalization and an intracellular processing event (①), after which the antigenic fragment is displayed with MHC class II molecules (Ia molecules) (arrow). The expression of IL-1 (②) by the macrophage is also required for the interaction. Microorganisms (—) stimulate IL-1 by themselves while isolated proteins (○) require the intervention of the CD4-positive T cell. Among the many proteins secreted by T cells is γ-IFN, which induces MHC class II molecules (③) on the macrophage and activates it for cytocidal function relevant for host defense. Interferon-γ may also be produced by non-T, non-B cells after microbial infections (upper part of the figure). T_h, helper T cell.

inflammatory reactions, and activation of CD8-positive cells to become active killer cells. (iv) The CD8-positive cell recognizes an antigenic determinant on a target cell, but the interactive or restrictive molecules are those of MHC class I.

What are the molecular events involved in the interaction between the APC and the CD4-positive cell—that is, during antigen presentation? What happens to the protein antigens when in the cell? How are the CD4-positive cells activated? Insights into these critical questions have come mostly from studies using macrophages, and also B cells, as APCs. For effective antigen presentation, the macrophage, or any APC, must have the capacity to (i) take up the antigen, internalize, and "process" it if necessary, (ii) express MHC class II molecules, and (iii) secrete growth-differentiating molecules such as IL-1, which are required to activate the T cell (Fig. 1). These three issues will now be considered.

Processing of Proteins by Macrophages

Processing to us means changing the protein so that it acquires an affinity for an MHC class II molecule. This can be done with some proteins by unfolding them and with others by partial proteolysis. Some proteins may not require treatment. The affinity of the processed protein or fragment for an Ia molecule is one factor that establishes the response to the protein.

The CD4-positive T cell is forced to recognize proteins on surfaces of cells bearing Ia molecules. Attention has turned recently to an analysis of the changes proteins may undergo after they are taken up by the macrophage. The results of many experiments in vivo on the response to globular protein antigens must be borne in mind. Starting with Gell and Benacerraf in 1959 (*9*), it became apparent that B cell responses (antibody production) were directed to "conformational determinants," which require the proteins to be in their native configuration and which involve amino acids from distant sites in the primary sequence (*9, 10*). In contrast, most T cell responses were directed at determinants found in the denatured protein. Moreover, in the case of the T cell, later studies established that the MHC could determine which portion of the protein was preferentially recognized, a process that Rosenthal called "determinant selection" on the basis of studies between macrophages and T cells in which insulin was used as antigen (*11*).

The first direct demonstration of an antigen processing event came from our studies. In order to follow the protein antigen in the macrophage, we developed a system in which the interaction of T cells and macrophages was examined within minutes. In our first experiments, we used a bioassay developed in our laboratory by K. Ziegler to examine the physical attachment of T cells to macrophages presenting the antigen (*12*). Using the bacteria *Listeria* monocytogenes, we established that antigen-specific T cells became attached to macrophages bearing MHC class II molecules only after the internalization of the bacteria. T cells did not bind at a time that the bacteria were on the macrophage surface or immediately after their internalization. Furthermore, macrophages that had phagocytized the bacteria and were then lightly fixed in formaldehyde could still serve as presenting cells. Thus light fixation did not completely denature the MHC class II molecules or the antigenic determinant. A second observation indicated that brief treatment of the macrophages with chloroquine or ammonium chloride impaired presentation. Chloroquine and ammonium chloride are weak bases that are concentrated in acid vesicles, raising their *p*H, and therefore affecting processes like catabolism. Thus the scenario that developed from the *Listeria* experiments was that the macrophage had to internalize the bacteria in an acid vesicular compartment for an immunogenic determinant to be displayed on the cell surface.

The next developments centered on the analysis of natural globular proteins. Most proteins followed the same steps as *Listeria*–namely, internalization and cycling through an acid compartment (*13–15*). Thus the results with *Listeria* were not limited to an apparently complex structure such as a microorganism. After the protein was internalized, it was displayed by the macrophage in forms ranging from a denatured molecule to small proteolytic fragments (*13*). This observation was based on studies with aldehyde-fixed cells and T cell hybridomas, which are used to probe the protein determinant after processing by the macrophage. The use of radioisotope-labeled protein gives limited information inasmuch as it is impossible to establish which of many peptides that escape lysosomal digestion are immunologically relevant. The T cell hybridomas are monoclonal T cells that react with a single determinant, or epitope, on an antigen molecule and that secrete lymphokines after presentation of the protein by a macrophage bearing an MHC class II molecule. Such T cell hybridomas are therefore valuable permanent cell lines for the study of recognition of protein. Hybridomas, however, have no growth control and therefore are not representative of T cells for the study of activation and metabolic events, as described in a later section. T cells are usually in a resting state until activated by antigen.

Recent studies have revealed the areas of a protein molecule that are recognized by the immune system. In our experiments with the protein hen-egg lysozyme (HEL), none of the T cells from mice immunized with native HEL recognized HEL on the macrophage surface unless the HEL was first internalized (*13*). The native HEL is a highly charged molecule that binds well to the surface of macrophages. When protein fragments were added to fixed cells, the T cells were able to recognize one of the fragments. This latter approach, first used by Shimonkevitz *et al.* (*14*) has now been used to determine which epitope in the protein is presented to the immune system. With HEL we found that the immunodominant epitope in H-2^k mice was included in a tryptic fragment 46–61 (Table 1). In the native HEL molecule, this sequence is partially buried in a β-pleated sheet structure. We have identified two sets of T cells that recognize HEL(46–61). One set reacts with HEL(46–61) presented by the macrophage as a small fragment, the optimal length being the ten amino acids of HEL(52–61); a second one reacts to the HEL(46–61) determinant when it is exposed in the denatured molecule.

The interaction of MHC II molecule with peptides to create the antigen determinant. Why do many proteins have to be processed for them to be immunogenic? We favor the hypothesis that intracellular processing selects for the portion of the molecule or epitopes that have an affinity for the MHC class II molecule and that the epitope associated with MHC class II molecules creates the determinant recognized by CD4-positive T cells. That the basis for the MHC restriction was an affinity of an MHC molecule toward an epitope of the protein molecule was championed by several immunologists, in particular by the laboratories of Benacerraf (*16*) and Schwartz (*17*). The alternative hypothesis was that the T cell recognized the two molecules—that is to say, the antigen and the MHC molecule—by two different receptors. Molecular and cellular immunobiologists have now identified a single receptor in the T cell. The development of the specificity of the T cell receptor to antigen plus an MHC molecule appears to take place in the thymus gland. There, early immature T cells are generated in close anatomical relation to epithelial cells bearing MHC class II molecules. Schwartz and his associates (*17*) argued strongly in favor of a contact area of the antigen, which they called an agretope, based on patterns of recognition of different cytochrome c molecules.

Direct evidence for the association of MHC class II molecules with proteins comes from our recent experiments in which purified

Table 1. The interaction of a lysozyme peptide with class II molecules. Peptide 1 (fragment 52–61) of hen-egg lysozyme (HEL) binds to MHC class II molecules of the murine I-A^k allele and activates a panel of T cell hybridomas (*18*). A substitution of Phe for Leu at residue 56 creates a peptide identical to that found in mouse lysozyme (peptide 2). This peptide does not stimulate the T cells directed to HEL(52–61) but binds to MHC class II molecules and competes for the presentation of peptide 52–61 to T cell hybridomas. We have assumed that the residue at position 56 is therefore not critical for binding but for contacting the T cell receptor. The same results apply to peptide 3. An Ala substitution at residue 61 results in a peptide (peptide 4) that will neither stimulate nor bind to Ia, nor will it compete for binding to Ia. The Arg at 61, therefore, may be involved in the contact with Ia molecules. An Ala substitution at residue 55 (peptide 5) will stimulate and therefore may not be critical for binding to the T cell nor to Ia. The explanation is in the text.

HEL peptide	Stimulation	Binding	Competition
52 61			
1. Asp-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg	+	+	NA
2. ----------Phe----------	−	+	+
3. ----Ala----------	−	+	+
4. ----------Ala	−	−	−
5. ----------Ala----------	+	+	NA

MHC class II molecules and the immunogenic peptides of HEL were studied in free solution (*18*). The MHC class II molecules bound the peptide HEL(46–61) in a saturable process with an affinity in the micromolar range (Table 1). Of key importance was the finding that HEL(46–61) associated only with those Ia molecules from responder and not nonresponder alleles. Some strains of inbred mice recognize primarily fragment HEL(46–61) of HEL, whereas others recognize other determinants of HEL. Only the former react immunologically when immunized only with HEL(46–61), whereas the latter are nonresponders. We have now found identical results with a second peptide from HEL, and Grey's laboratory has confirmed and extended these studies with other peptides (*19*). Undoubtedly the interactions between Ia and proteins have to be explored further. Some have argued that the association takes place best when the T cell itself with its receptor stabilizes an initial weak interaction (*20*).

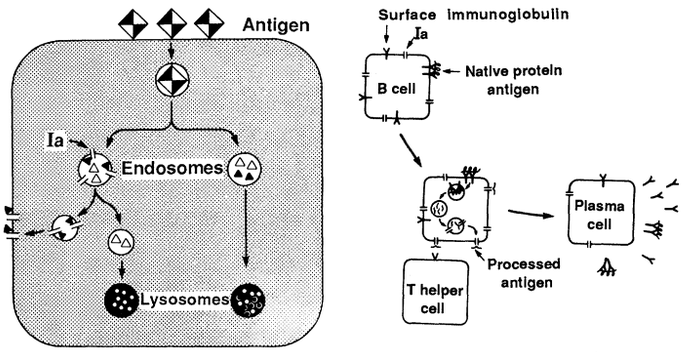
Further evidence that the functional role of Ia molecules is to interact with the antigen comes from experiments on antigenic competition. When two antigens are administered at about the same time, one of the antigens may inhibit the response to the other. Antigenic competition takes place only with protein antigens and not with polysaccharides, which trigger limited activation of B cells without the involvement of T cells. Antigenic competition can take place at the level of the presenting cell and only among peptides that are presented in the context of the same MHC class II molecule (*21*). We found that the only peptides that competed for the binding of HEL(46–61) to Ia were those presented by the same Ia molecule (*18*). The peptides that competed for binding also competed for functional presentation to T cells. In our studies, we used derivatives of the HEL(46–61) peptide to map the contact residues for the MHC class II molecule (Table 1). For functional presentation, we used macrophages treated with the HEL peptides and lipid monolayers containing only Ia molecules. Our data indicated that antigenic competition took place during the interaction of MHC class II molecule with peptide. Three important results were noteworthy. (i) One of the peptides that competed for the binding was an autologous peptide of lysozyme, an indication that the Ia molecules do not discriminate between self and nonself. [Since the original description of Ir genes and Ia molecules, a controversy has arisen as to which cell—the APC or the CD4-positive cell—is responsible for the lack of response to a given peptide antigen (*22*). The data on the binding of peptides to selected MHC class II molecules support the explanation that the APC is the responsible cell. However, other data would indicate that sets of antigen-reactive T cells could be inactive or absent—referred to as the "hole in the repertoire" (*22*). We believe that peptide-Ia interaction is a necessary but insufficient condition for immunogenicity. Our finding that autologous lysozyme peptides bind to self Ia molecules would indicate that a second mechanism can exist. In this example, it is clear that the dormancy of the CD4-positive cells that recognize self-lysozyme is required to avoid autoimmunity.] (ii) Native HEL did not compete with HEL(46–61) for binding to Ia, an indication that the protein in its native state may not interact with Ia molecules. (iii) Unrelated peptides presented by the same MHC class II molecule competed for binding and presentation, evidence that the MHC class II molecule had a single functional binding site (*19, 23*).

The experimental data support the view that the epitope recognized by T cells is created when the protein interacts with the Ia molecule. The peptide, when it binds to the Ia molecule, may develop unique conformational features recognized by the T cell system. The different alleles of the MHC class II molecules have areas of hypervariability that can form the combining site for peptides (*6*). In our view, the Ia polypeptides will have a broad specificity of recognition, with affinities ranging from the micromolar to the millimolar range. This affinity is one factor that dictates whether peptides have a chance of being presented to the immune system.

The biochemical basis of the association between Ia and proteins is under current analysis. The initial indications are that the immunogenic peptides have contact residues for Ia interspersed with other residues that interact with T cells. We have been able to dissect which amino acid residues contact the Ia molecule and which contact the T cell receptor in the peptide 52–61 of HEL; this is the shortest peptide recognized by our clones (Table 1). Three residues, two of which, Asp^{52} and Arg^{61}, are charged, and one of which, Ile^{58}, is hydrophobic, contact Ia; three other residues, Tyr^{53}, Leu^{56}, and Gln^{57}, contact the T cell. The side chain of the four remaining residues are not involved in either function and appear to serve as spacer residues. Computer modeling shows that if HEL(52–61) is placed in an α-helix, the side chains of the residues that contact the T cell and those that contact Ia are segregated on opposite sides of the helix. This allows the residues that contact the T cell to be exposed for recognition by T cells (*24*). Arguments in favor of such an α-helical structure or for an amphipathic α-helix have also been proposed (*25*). The definition of an antigenic determinant for the T cell is in its early stages, but these results are encouraging, particularly in the perspective of using defined determinants for prophylactic immunization. So far there are no striking sequence homologies among peptides that interact with a given Ia molecule. They may have in common the property of forming stable secondary structures when in association with the Ia molecule.

These early studies have raised many new questions and opened new perspectives. One important question is whether all proteins need to be processed for their putative interaction with Ia molecules. One of us (P.M.A.) has just studied human fibrinogen as an antigen and concluded that, in contrast to most other proteins, it does not require processing. The requirements for processing may depend on the conformational freedom of the antigen molecule to interact with Ia.

Fig. 2 (**left**). Our view of the cellular events that result in a display of an immunogenic protein determinant. A globular protein is first taken into an acid vesicular compartment that bears Ia molecules (endosome); there it is denatured and partially fragmented. Those fragments that have an affinity for Ia bind to it and are transferred to the cell surface while those that do not end up in lysosomes and are degraded to amino acids (arrows to the left). If the endosome does not bear Ia, the entire fragments end in the lysosomes (arrows to the right). This scheme explains the observations discussed in the text. **Fig. 3** (**right**). Antigen presentation by B cells explains the observations that B cell responses are directed to conformational determinants, whereas T cell responses are against sequences of amino acids. The explanation is in the text.



A second major question is at the level of the cell. Where in the macrophage do Ia and antigen interact? There is no doubt that some Ia molecules can be localized in intracellular vesicles. We found Ia in the phagolysosomes and more recently Creswell (*26*) demonstrated that exogenous molecules can establish contact with intracellular Ia molecules. A model that we favor is that the protein enters an acid prelysosomal vesicular compartment where it is subjected to denaturation or partial fragmentation, or both. This endosomal vesicle contains newly synthesized or recycled MHC class II molecules. Those products of processing with affinity for the MHC class II molecule interact with it and are then transported to the plasma membrane for presentation. Those that do not associate will be targeted to lysosomes for extensive breakdown (Fig. 2). The Ia system functions then as a carrier system that protects peptides from catabolism and transports them to the surface. It is of interest to speculate whether the Ia molecules have this function for natural peptides outside the immune system.

A third key question regards our own observations that autologous peptides compete with immunogenic peptides and that Ia molecules do not have the fine specificity for recognition between self and nonself molecules. How does immunization then take place in face of an expected large mass of autologous products resulting from normal intracellular digestion? Immunization may be the act of overriding natural antigenic competition by producing changes in the antigen molecule that enhance uptake by the macrophage (such as by forming large polymers or aggregates).

Antigen Presentation by Other Cells

Although the studies that led to our present understanding of processing and presentation were mostly with macrophages, it now has become clear that antigen presentation is not an exclusive property of this cell. B cells, for example, have been shown convincingly to present antigen (*27*). The logical sequence for the interaction of B cells and T cells is that B cells select a protein by use of a membrane-bound antibody, internalize the protein, and process it in a manner that is analogous to the action of the macrophage (Fig. 3). The CD4-positive cells then recognize the antigen on the B cell surface in a way analogous to the way they recognize antigen on a macrophage. Recognition then leads to B cell activation. This sequence would explain the earlier observations on the differences between recognition of antigen by B cells and T cells. B cells can react with the protein antigen in its native state, and the selected clones would be those that have high affinity receptors for the antigen. Such B cell clones, after presentation of the internalized protein to T cells, would differentiate and secrete antibodies of the same specificity as the receptor immunoglobulin. Thus, B cell reactivity (that is, antibodies) can be against the protein in its native configuration, whereas reactivity of the T cell is against the processed protein.

Of interest are the results showing that L cells do not present protein antigens because they do not express MHC class II molecules (*28*). However, after gene transfection, these molecules can present antigen. The results with B cells, L cells, and others, therefore, tell us that intracellular protein processing is a generalized cellular property. Other APCs include the Langerhans-dendritic cells, which bear high levels of Ia molecules and are highly active in transplantation reactions (*29*). How exactly APCs are interrelated in immunologic reactions in vivo still has to be determined.

Control of Expression of MHC Class II Molecules

The restriction on T cell recognition, and therefore of the entire cellular immune system, imposed by the Ia molecules fulfills two purposes: (i) it assures that the species will be capable of reacting with many amino acid sequences inasmuch as the MHC gene loci are very polymorphic, and (ii) it controls T cell reactivity so that it takes place only on selected cells—namely, the APC cell family, which includes the macrophage. It now appears that the activation of CD4-positive T cells is regulated by controls placed on the macrophage and the APC system. One control is at the level of the Ia molecule. The second control mechanism is at the level of expression of IL-1, a molecule also required for T cell activation (Fig. 1).

The control of the activation of CD4-positive T cells may be especially critical for autoimmunity. Autoreactive B and T cells arise during normal development—as do those against foreign antigens—by random assembly of the gene segments that code for their receptors. Mechanisms must exist, therefore, to control antigen presentation and ensure that the autoreactive cells remain inactive. Our understanding of the control of MHC class II molecules and IL-1 is starting to emerge.

Macrophage activation. The most informative studies on the control of Ia expression have been made with the macrophage. Experiments using the mouse indicate that macrophage Ia expression is not constitutive but is under regulation (*3, 30*). Some of the mouse monocytes and young macrophages, depending on the tissue, express Ia molecules. This basal expression takes place when the immature phagocyte stops proliferating. Early cells of the macrophage lineage proliferate in the marrow and also in tissues; the development of many macrophage traits takes place when proliferation stops and the cell then matures. The mature macrophage will not proliferate further. The expression of Ia is transitory and eventually all Ia-positive macrophages become Ia-negative and therefore lose their antigen-presenting properties. Ia expression is not a marker for a stable subset of macrophages. A clear example of the interaction of macrophages with their tissue environment is found in the ratio of Ia-positive to Ia-negative macrophages, which varies greatly among different tissues. For example, macrophages in

spleen red pulp are mostly Ia-positive, whereas those in the white pulp are mostly Ia-negative. Peritoneal macrophages are mostly Ia-negative. In most tissues the basal ratio of Ia-positive to Ia-negative macrophages is independent of T cells. For example, athymic mice or mice with the severe combined immunodeficiency mutation have normal ratios of Ia-positive to Ia-negative macrophages (*31*). The reasons for the great differences in this ratio among tissues is not well established. One factor that influences this ratio is the local level of prostaglandins, which at nanomolar concentrations inhibit Ia expression (*32*). For example, the peritoneal macrophage spontaneously produces large amounts of prostaglandins and, in fact, limiting this production (by drug treatment) results in severalfold increases in the basal level of Ia-positive macrophages. This is a situation in which the macrophage regulates its own expression of a key molecule required for its function. Prostaglandin production appears to vary, however, among different macrophage populations, although this issue is yet to be completely studied. The monocyte, for example, is a very low producer.

The only established inducer of Ia expression known at present is γ-IFN (*33*). Interferon-γ is produced by T cells during antigen presentation (Fig. 1). This molecule binds to macrophages and induces new expression of messenger RNA for Ia molecules. Therefore, shortly after antigen presentation there is a rapid production of γ-IFN with high levels of Ia expression on the macrophage. This reaction is impressive in microbial infections. For example, an intraperitoneal infection with *Listeria* monocytogenes resulted in a rapid migration of blood monocytes, which, as mentioned, are low producers of prostaglandins and rapidly develop Ia. By 3 to 5 days, as γ-IFN was produced, the number of Ia-positive macrophages reached about 100 percent from an initial basal level of 5 to 10 percent. Such macrophages were highly active in antigen presentation when tested in culture. The γ-IFN–treated macrophages also exhibited cytocidal function upon their interaction with other stimuli (called "second signals") of which bacterial products like endotoxin are the most prominent (*34*). This is the best example of the close reciprocal interaction between macrophages and T cells. After antigen presentation, in which the bacteria has to be processed by the macrophages, the T cell is activated and produces γ-IFN; this induces an inflammatory reaction in which the activated macrophage is prominent. The whole process subsides as the antigen is eliminated. The continuous activation of the T cell is dependent on repeated exposure to antigen.

The expression of Ia molecules brought about by γ-IFN is not limited to macrophages but extends, under prolonged antigenic stimulation, to many cells like epithelial, endothelial, and connective tissue cells (*35*). These cells with their newly acquired Ia have the potential to present antigen and to interact with T cells. Perhaps such aberrant Ia expression may be one factor that results in the stimulation of autoreactive T cells directed to self proteins to cause autoimmunity (*36*). Although there is no formal proof of this, the fact that Ia can be detected in tissues like thyroid and pancreatic islets undergoing immunological reactions is very provocative. Continuous microbial infection with prolonged synthesis of γ-IFN could conceivably be the cause of systemic expression of Ia molecules.

Interleukin-1

Some important interactions between the immune system and the macrophage involve IL-1 (Fig. 1). IL-1 has a dual role: as a mediator of immunological cellular interactions during antigen presentation, which we believe most likely involves a membrane form of IL-1; and as a hormone that modulates tissue responses in inflammation, which requires the secreted form. IL-1 was discovered as a product released by endotoxin-treated human monocytes required for the growth response of thymocytes to the plant lectin phytohemagglutinin (*37*). Thymocytes, in contrast to T cells of lymph nodes and spleen, normally proliferate in the thymus but stop proliferating as soon as they are removed from the gland and placed in culture. They proliferate again if IL-1 and a second stimulus such as that provided by the plant lectins concanavalin A or phytohemagglutinin are added. Studies with complementary DNA clones from human and murine cells have revealed two distinct IL-1 molecules termed IL-1α and IL-1β (*38*). Both forms are made as a precursor molecule of about 30 kD, which is later processed to a smaller bioactive product of about 17 kD. IL-1α and IL-1β have 25 to 40 percent amino acid sequence homology, depending on the species. Initial studies indicate that the two forms have identical activities and bind to the same receptors (*39*).

Although IL-1 is produced in large amounts by the macrophage it is not an exclusive product of this cell (*40*). IL-1 is secreted by both lymphoid and nonlymphoid cells, although it is not known whether all IL-1 activities reside in identical molecules. IL-1 production may, therefore, be part of a general response to stress and inflammatory signals.

The broad range of bioactivities regulated by IL-1 attests to its having a fundamental role. All indications favor IL-1 as being an important component in the activation of T cells during antigen presentation. However, whether all T cells, regardless of their state of activation, require IL-1 is still undecided. IL-1 acts on T cells in two ways: it induces receptors for interleukin-2, which would then allow the T cell to respond to this T cell growth factor; it also stimulates interleukin-2 production (*41*). Not only does IL-1 promote growth of lymphoid elements but it has effects on many cells and tissues including the liver, brain, connective tissue, muscle, bone, pancreatic islets, and neutrophils (*40*). IL-1 is one of the major molecules released into the circulation after infection and responsible for fever. It induces hepatocytes to release acute phase reactants, makes endothelium adhesive for monocytes, promotes growth of fibroblasts, increases bone resorption, and induces muscle wasting. The modulatory role of IL-1 in promoting local inflammation can be clearly inferred. As more and more effects of IL-1 are identified, the possibility arises that this molecule is involved in the pathogenesis of inflammatory or degenerative diseases. IL-1 has been mentioned in the context of rheumatoid arthritis, osteoporosis, pulmonary fibrosis, and insulin-dependent diabetes mellitus. In essence, IL-1 may trigger essential metabolic processes that are expressed in different ways by the target cells. The exact biochemical pathways generated by IL-1 are not well worked out, and more definitive work is urgently needed. IL-1 is one of several important products secreted by macrophages. There has been recent interest in tumor necrosis factor or cachectin, a protein released by macrophages during severe infection and which affects diverse cells, including neoplastic cells (*42*). This molecule, however, in contrast to IL-1, does not stimulate lymphocytes.

Our own interest in the role of IL-1 as a protein that regulates T cell activation concerns a membrane-associated form. We were struck by our finding that formaldehyde-fixed macrophages not only presented protein antigens to T cells, but stimulated their growth and differentiation. Either IL-1 was not involved or it was present as a membrane component resistant to the aldehyde fixation. Isolation of membranes led E. Kurt-Jones, working in our laboratories, to recognize a bioactive membrane-associated IL-1 (mIL-1) that was resistant to aldehyde fixation and behaved physically as an integral membrane protein (*43*). Since antibodies to IL-1 neutralized antigen presentation by fixed macrophages, it became clear that mIL-1 had a role in T cell activation following recognition of MHC class II

molecules and antigen. The biochemical nature of mIL-1 and its relation to secreted IL-1 or intracellular IL-1 are not definitely established. We do not know how IL-1 is bound in the membrane. It has a size of approximately 18 kD, it is solubilized by detergents and neutralized by antibodies to IL-1. There is an absence of hydrophobic or signal sequences in the cloned cDNA in both forms of IL-1. These observations raise the issue of how the protein is secreted or placed on the membrane.

Is IL-1 expression regulated? Phagocytes freshly isolated from liver, spleen, blood, or the peritoneal cavity do not express mRNAs for IL-1α or IL-1β, nor do they show intracellular IL-1 or membrane IL-1 (*44*). There are two sets of immunological stimuli relevant for IL-1 expression: one set of stimuli comprises those that directly induce IL-1 via the macrophage (*40*) and includes a variety of microbes and their products and also some immunological adjuvants (substances that nonspecifically enhance immunity, including, for example, the Freund's type of adjuvant and some simple compounds like beryllium sulfate); a second set comprises isolated proteins and peptides that induce IL-1 indirectly via the CD4-positive T cell, another example of the close reciprocal interaction between macrophages and T cells (*45*) (Fig. 2). When the protein is processed and the immunogen is displayed on the cell surface together with an MHC class II molecule, the T cell recognizes the complex and induces IL-1 on the macrophage. Depending critically on amounts of antigen and class II molecules displayed, the macrophage is induced within a few hours to produce mIL-1. We have shown that both cell contact and a T cell–secreted product are responsible (*45*). The T cell product has yet to be characterized but includes two activities—one that acts on the macrophage and a second distinct one that acts on B cells. Once antigen, the MHC class II molecules, and IL-1 are available, the CD4-positive T cell responds in a cascade of activation steps that include the secretion of products like γ-IFN that act back on the mononuclear phagocyte system. Finally, the possibility that other controls on antigen presentation besides those exerted by Ia and IL-1 expression is being explored. It would not surprise us if many factors come into play.

We have examined the immune system as it relates to the macrophage. The study of the interaction of macrophages and lymphocytes has added to our basic understanding of host defense and immunity and has led to insights into the nature of cell-cell communication as well as into issues of intracellular handling of protein and the function and significance of histocompatibility. A major message is that macrophages and lymphocytes require each other to function and that specific and nonspecific immunity are closely related.

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