

Basic Concepts of Immune Response and Defense Development

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Abstract

The induction of immune responses requires critical interaction between *innate* parts of the immune system, which respond rapidly and in a relatively nonspecific manner, and other *specific* parts, which recognize particular epitopes on an antigen. A critical element in this interaction is the role played by dendritic cells (DCs), which represent “professional antigen-presenting cells.” DCs endocytose and process antigen to peptide presented on the cell surface in association with major histocompatibility complex (MHC) molecules. This presentation results in interaction with and stimulation of helper T (Th) lymphocytes, which recognize peptide in association with either MHC class II or cytotoxic T (Tc) lymphocytes, which recognize peptide in association with MHC class I. Stimulation of Th lymphocytes produces the growth and differentiation factors (cytokines) essential for the B lymphocytes that have responded to a more intact form of the antigen and that differentiate into antibody-producing cells. The precise interaction between the cells depends on cognate ligand-receptor recognition between the B and Th lymphocytes. DCs also play a direct role with the stimulation of the B lymphocytes. It appears that DC can deliver antigen to the B lymphocytes in a more intact form than the processed form essential for stimulating T lymphocytes, and can release cytokines that assist the differentiation of the B lymphocytes into antibody-producing cells. This close relationship among the three cell types and the cytokines that are produced ensures the precise control and regulation necessary for immune response development.

Key Words: antigen presentation; cytokines; dendritic cells; immune defenses; lymphocyte responses; vaccine design; vaccine targets

Introduction

The defense of the body against physical and event-related attacks on its well-being depends on its immune system. For example, in response to an attack by infectious agents, the immune system must destroy the “dan-

ger” and maintain the health of the body. The problem with many infectious diseases is that the response of the immune system occurs *after* invasion of the organism, hence disease symptoms have already appeared. One approach that circumvents such a problem and assists the immune system is to vaccinate with an innocuous form of the disease-producing agent, which is still capable of simulating the immune system. This approach results in immune defenses that protect the body against future attack by the pathogen.

The elements that define a successful immune defense against an infectious disease are the immunological correlates of protection. Immune defenses develop after critical interaction between the two main parts of the immune system—innate and specific. Innate defenses are characterized by rapid assimilation, which is particularly valuable in an emergency situation, and nonspecific response, which is of limited duration (Janeway and Medzhitov 2002; Medzhitov and Janeway 2000a). These characteristics include humoral factors (e.g., mucosal secretions) and serum factors (including complement, certain cytokines, and natural immunoglobulins). The Cellular components of innate defenses include natural killer (NK¹) cells, granulocytes, macrophages (MΦs¹), and dendritic cells (DCs¹). Specific immune responses are longer in developing but are specific and more durable. To be efficient, these specific defenses require interaction with innate parts to develop (Medzhitov and Janeway 1999). With both immunizations and vaccinations, adjuvants are often employed to enhance the development of an efficacious specific response. Many of these adjuvants act on the innate responses. Examples are lipopeptides and cytosine-phosphorothioate-guanine oligode-

¹Abbreviations used in this article: APC, antigen-presenting cell; BCR, B cell receptor; CLIP, class II-associated invariant chain peptide; CpG, cytosine-phosphorothioate-guanine; CpG-ODN, cytosine-phosphorothioate-guanine oligodeoxynucleotide; DC, dendritic cell; DriP, defective ribosomal product; ER, endoplasmic reticulum; FcγRII, receptor type II for the Fc portion of immunoglobulin G; FcγRII, receptor type III for the Fc portion of immunoglobulin G; GACP, granule-associated cytotoxic protein; iDC, immature dendritic cell; IFN, interferon; Ii, invariant chain; IL, interleukin; MΦ, macrophage; MIIC, major histocompatibility complex class II-rich part; mDC, mature dendritic cell; MHC, major histocompatibility complex; NIPC, natural interferon-producing cell; NK, natural killer; ODN, oligodeoxynucleotide; PAMP, pathogen-associated molecular pattern; Tc, cytotoxic T; TCR, T cell receptor; TGF, transforming growth factor; Th, T cell helper; TLR, Toll-like receptor; TNF, tumor necrosis factor; Treg, regulatory T lymphocyte.

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oxynucleotide (CpG-ODN¹)-motifs, which stimulate cells such as DCs through Toll-like receptors (TLRs¹) (Diefenbach and Raulet 2003; Gordon 2002; Netea et al. 2004).

The critical interaction between the innate and specific parts of the immune system involves the role played by antigen-presenting cells (APCs¹), which include monocytes, MΦs, endothelial cells, fibroblasts, fibrocytes, and DCs (Guermontprez et al. 2002; Knight and Stagg 1993). From the specific immune part, B lymphocytes can also function as APCs, but not for stimulating naive lymphocytes in primary immune responses. Because DCs are at the center of antigen presentation for both primary and recall immune responses, they are referred to as “professional APCs.” Although DCs are particularly important APCs, not all monocytes and MΦs behave as APCs. MΦs are often more involved in effector immune responses due to their “scavenger” phagocyte function (McCullough et al. 1992), and they can even suppress the development of antigen-specific responses (Basta et al. 2000).

When an immune system responds for the first time to an infectious agent, or in fact to any material regarded as foreign to the body, the efficiency of that response depends on the involvement of the DCs (Gatti and Pierre 2003; Guermontprez et al. 2002; Mellman and Steinman 2001). This activity comprises the primary immune response. The efficiency with which DCs function as APCs, the fact that this appears to be their principal function, and their necessary role in initiating primary immune responses have resulted in DCs being referred to as professional APCs. In addition to the APCs from the innate part, B lymphocytes can also present antigen in association with MHC molecules to T lymphocytes (Finkelman et al. 1992). The response of the B lymphocyte differs from the APCs of the innate part in that B lymphocytes tend to present antigen only to T lymphocytes that have already responded to antigen—the so-called memory T lymphocytes.

The function of APCs is to present the antigen from the invading infectious agent to the B and T lymphocytes that possess specificity for the epitopes on that antigen. B lymphocytes interact directly with the antigen, via their cell surface “B cell receptor (BCR¹),” to differentiate into cells that produce antigen-specific antibody. To accomplish this differentiation, the B lymphocytes must interact with antigen-specific T lymphocytes—specifically the T helper (Th¹) lymphocytes. Th lymphocytes produce the necessary factors (cytokines) and intercellular communications (cell surface costimulatory molecules) to drive antigen-stimulated B cells into differentiation that results in antibody-producing plasma cells. Unlike the B lymphocytes, T lymphocytes cannot interact directly with antigen; they must process the antigen into small peptides presented in association with MHC molecules. The APCs are responsible for processing the antigen and associating the derived peptides with the MHC molecules. These peptide-MHC complexes are presented on the surface of the APC, hence the term “antigen presentation.” The T cell receptor (TCR¹)

on the T lymphocytes can recognize only the peptides derived from this processed antigen in association with MHC.

Innate Immune Defenses

Innate immune defenses are composed of numerous and variable components. The skin, mucosal secretions, and stomach and intestinal pH are physical/chemical barriers that form the first line of innate defense (Bals 2000; Fellermann and Stange 2001). When an infectious agent evades these barriers and invades the host, cellular innate immune defenses come into play. The agent first encounters histiocytes and monocytic cells (Hoffmann et al. 1999; Medzhitov and Janeway 2000a). Other cells of the innate immune defenses include the NK cells, which attack infected host cells (Biron et al. 1999) and mast cells involved in anaphylactic-type responses (Feger et al. 2002). Although lymphocytes are generally regarded as part of the specific immune system, NK cells are lymphocyte-like in humans, and a subset of T cells—the $\gamma\delta$ T lymphocytes—can interact with antigens in a more “innate” manner (Boismenu and Havran 1997) (see below). As the first elements of the immune system to encounter an invading agent, innate defenses are activated more rapidly than the specific responses but are of shorter duration.

Although cellular defenses of innate immune responses do not recognize specific epitopes on an antigen in the manner observed with the specific immune responses, they do rely heavily on the interaction of particular cell surface receptors with the pathogen in question (Gallucci and Matzinger 2001; Gordon 2002). Such receptors are pattern recognition receptors, referred to as pathogen-associated molecular patterns (PAMPs¹) (Beutler 2000; Fraser et al. 1998; Gordon 2002; Medzhitov and Janeway 2000b). Examples are the TLR family (Medzhitov and Janeway 2000b; Takeda and Akira 2001; Vasselon and Detmers 2002), the complement receptors (Gasque 2004), the mannose receptor (Fraser et al. 1998; Stahl and Ezekowitz 1998), and CD14 (Triantafilou and Triantafilou 2002). C-type lectins, receptors for heat-shock proteins, and certain integrins are also important (see Théry and Amigorena 2001), although integrins are involved in regulating cellular interactions during both innate and specific responses.

Innate responses comprise a number of soluble factors. These factors include the serum proteins, which can bind to the surface of the invading agents and are called opsonins. Examples are complement, natural antibodies, lipopolysaccharide-binding protein, mannan-binding protein, and acute phase proteins. The latter, as well as certain by-products of the complement activation (complement cascade or factors), also play a signaling role, as do the following: chemokines; innate defense cytokines (particularly interleukin [IL¹]-1 β , IL-6, IL-8, IL-10, IL-12, IL-15, tumor necrosis factor [TNF¹]- α , transforming growth factor [TGF¹]- β , interferon [IFN¹]- $\alpha/\beta/\gamma$); histamine; prostaglandins; and leukotrienes (Barrington et al. 2001; Biron et al. 1999; Matsushita et al. 1998; Rossi and Zlotnik 2000).

Activation of Innate Immune Defenses

Some local tissue damage occurs after immunization or infection and results in the release of exogenous immune response mediators referred to as “danger signals” (Gallucci and Matzinger 2001). These mediators interact with histiocytes (MΦs and neutrophils), DCs, and mast cells at the site of injury or vaccine deposition, and result in the production of endogenous mediators that promote immune defense system recruitment through the local inflammatory immune reaction. An important element in this process is the local increased endothelium permeabilization, concomitant with upregulation of integrin and other adhesion molecule expression on the endothelium. Chemokines released from the endothelium recruit histiocytes and other leukocytes from the blood to adhere to the endothelium with modulated adhesion molecule expression (Mackay 2001; Murdoch and Finn 2000). The increased binding at the endothelium permits extravasation of the recruited leukocytes between the junctions of the endothelium into the site of injury. This recruitment of leukocytes is due to chemotaxis, and is a key element in orchestrating selective recruitment of leukocytes to inflammatory sites.

Neutrophils and monocytes, including DCs, typically dominate migratory leukocytes in innate defense responses (Murdoch and Finn 2000). The mononuclear phagocytes (monocytes and MΦs) and neutrophils are central to the acute inflammatory reaction of innate effector immune defenses. They are important immune defense agents because they are actively phagocytic and have a wide distribution throughout the body tissues and organs. By comparison, the DCs, which are recruited to the site of antigen presence, are primarily involved in antigen processing and presentation to antigen-specific lymphocytes. For this activity to occur, the DCs must migrate from the site of antigen uptake to the lymphoid organs and tissues to interact with the antigen-specific lymphocytes (Gunn 2003; Randolph 2001). Thus, DC uptake of antigen results in the transport of that antigen to the lymphoid organs and tissues wherein specific responses develop.

Interaction of the Innate and Specific Parts of the Immune System

DCs at the local inflammatory site include dermal DCs and Langerhans cells. In addition, DCs referred to as “immature DCs” migrate from the blood into the inflammatory site. Together, they play an important role in linking the innate with the adaptive immune system. DCs at the mucosal surfaces, including the mucosal-associated lymphoid tissue (“MALT”) and tonsils, are particularly important in terms of how they interact with foreign material (Holt et al. 1999; MacPherson and Liu 1999). The latter can be transported across or through the epithelial barrier at these sites, and passively taken up from the epithelial cells by DCs. In addition, there is evidence that local DCs can open the tight junctions between epithelial cells at mucosal surface barriers

in a way that retains the integrity of these junctions (Bozza et al. 2002; Rescigno et al. 2001a,b). This capability permits the DCs to send a cellular protrusion into the lumen at the mucosal surfaces, “capture” the foreign material, and transport it into lymphoid tissue for presentation. Once in the lymphoid tissues or organs, the DCs enter follicles to deliver antigen to the B lymphocytes and to present processed antigen in association with MHC molecules to T lymphocytes (Banchereau and Steinman 1998; Banchereau et al. 2000). As a result of this process, specific immune responses are induced.

Innate-specific Interactions: Antigen Processing/Presentation

Although monocytes and MΦs had been considered as APCs of the immune system, the principle APCs are the DCs (Steinman 1991), which are found in all lymphoid tissues, the blood, lymphatic circulation, and nonlymphoid tissues. The main function of DCs is antigen presentation (Banchereau et al. 2000; Steinman 1991), in contrast to other cells that can function as APCs (e.g., as MΦs and B cells). DCs are actually a heterogeneous population of cells, as exemplified by recent studies in swine (Summerfield et al. 2003). Nevertheless, one consistent element is the “maturation” of DCs during antigen processing/presentation. DCs are compartmentalized into immature DCs (iDCs¹) and mature DCs (mDCs¹) (Romani et al. 1989; Schuler and Steinman 1985). Langerhans cells and dermal DCs, which are good examples of iDC, seek out antigenic material for endocytosis that leads to processing and presentation. Once iDCs endocytose and commence the processing of antigenic cargo, they begin to mature (Banchereau et al. 2000; Pulendran et al. 2001). Inflammatory “danger” signals, including proinflammatory cytokines and factors released during tissue damage (e.g., uric acid) promote this maturation (Shi et al. 2003). Microbial exogenous signals can also induce maturation through the PAMPs described above (Fraser et al. 1998; Gordon 2002; Medzhitov and Janeway 2000b). It is important to consider these elements during the formulation of vaccines.

DC Presentation of Antigen to Th Lymphocytes

DCs can internalize antigen, or cells such as tumor, virus-infected, and apoptotic cells. Internalization is achieved either by constitutive macropinocytosis of intracellular fluid (“environmental sampling”) or binding to one of the numerous receptors possessed by DCs (Théry and Amigorena 2001). When iDCs endocytose antigen, the uptake can use one of the different forms of endocytosis—clathrin-dependent endocytosis, macropinocytosis, pinocytosis, or phagocytosis—depending on the size and nature of the antigen or cell to be internalized. The internalized material is degraded into peptide fragments within the endosomal sys-

tem for processing that involves MHC class II molecules, or is transferred to the cytosol for processing that involves MHC class I molecules (Guermónprez et al. 2002; Steinman et al. 1999; Théry and Amigorena 2001). The MHC class II-dependent presentation of antigen is essential for stimulating the Th lymphocytes (see below).

Antigenic peptide-containing endosomal structures fuse with specialized structures that contain MHC class II molecules, the so-called MHC class II-rich part (MIIC¹). In human iDCs, newly synthesized MHC class II molecules carrying the invariant chain (Ii¹) first appear on the cell surface, from where they are internalized and targeted to the endosomal part through signaling via the cytoplasmic tail of the Ii (Théry and Amigorena 2001). The Ii is cleaved by cathepsin S, leaving what is referred to as the class II-associated Ii peptide (CLIP¹). In the MIIC, the antigenic peptides are catalytically exchanged for the CLIP, which allows interaction of the antigenic peptide with the peptide binding cleft of the MHC class II molecules that is capable of “recognizing” the peptide amino acid sequence. These peptide-loaded MHC class II molecules are then transferred to the cell surface, where they are stably expressed—an important characteristic of mDC. MHC class II molecules that lack peptide loading are not stably expressed on the cell surface but are continually recycled between the surface and the MIIC or are degraded by lysosomes.

The process described above has been observed with human DCs. However, with murine iDCs, newly synthesized MHC class II molecules can be retained in the lysosomal part and do not reach the cell surface until they are loaded with antigenic peptide (Théry and Amigorena 2001). Furthermore, not all MHC class II alleles behave identically. With mice at least, the cellular localization and peptide loading characteristics of MHC class II molecules depend on their haplotype (Théry and Amigorena 2001). In addition, with both human and murine DCs, the MHC haplotypes determine the amino acid sequence of the peptide to be recognized. The requirement for MHC-peptide interaction introduces a limitation in the number of processed peptides that can be presented by APCs, due to the MHC haplotypes. Antigen presentation depends on the alleles in the loci encoding the MHC class I or II binding site of the APCs. Individuals show a variation in their peptide recognition patterns through the possession of different alleles, due to differences in the MHC molecule “recognition” of the peptides (Zinkernagel and Doherty 1997, 1979)—referred to as MHC restriction. Polymorphism in the MHC class II loci leads to species-specific and individual-specific repertoires for the efficient recognition of antigenic peptides by CD4⁺ T lymphocytes.

Despite the differences described above, efficient antigen processing and presentation will result in the stable expression of peptide-loaded MHC class II molecules on the mDC surface. This stability of expression is critical because it facilitates the presentation of the molecules to the TCR on Th (CD4⁺) lymphocytes.

DC Presentation of Antigen to Cytotoxic T (Tc¹) Lymphocytes

MHC class I molecules also bind intracellular peptides and transport them to the cell surface, for presentation to Tc lymphocytes (Brode and Macary 2004; Heath et al. 2004; Théry and Amigorena 2001). This process is critically important in defending against intracellular pathogens such as viruses and certain bacteria. The Tc lymphocyte responses are also essential in the immune defense against tumors due to their role in recognizing antigens presented in the context of the host’s own MHC class I—the so-called “altered self.” For the initiation of such responses, pathogen or tumor cell antigens must be processed by APCs for presentation on their surface in the context of the MHC class I molecules. Again, it is the DCs that are the most potent at performing this function of antigen presentation (Guermónprez et al. 2002). As with antigen presentation in the context of MHC class II to Th lymphocytes, the additional involvement of costimulatory receptors is required to promote the interaction between the DC and the Tc lymphocytes. The DCs can express MHC class I molecules that carry the peptide antigen in question at sufficiently high levels, along with the required costimulatory molecules, to stimulate naive Tc lymphocytes.

For stimulation of Tc lymphocytes by DCs, the antigen must be processed in the DC cytoplasm. Although this requirement may appear similar to that involving MHC class II, the similarity ends there. The site of processing differs for MHC class II- and MHC class I-dependent presentation. For MHC class I presentation, many of the antigens processed into this pathway are referred to as endogenous, because the origin of the peptides interacting with the MHC Class I molecules are derived from the host cell or intracellular pathogens. In the majority of cases, these antigenic peptides are generated by the proteolytic cleavage of misfolded proteins, collectively termed “defective ribosomal products” (DRiPs¹) (Brode and Macary 2004). Following DC activation, DRiPs accumulate in cytosolic aggregates termed “dendritic cell aggresome-like inducible structures” (“DALIS”) (Lelouard et al. 2002, 2004). Ubiquitination of these complexes generates substrates for the cytosolic proteasome, which cleaves them into small peptides for transportation into the lumen of the endoplasmic reticulum (ER¹) by the transporter associated with antigen presentation (“TAP”) (Brode and Macary 2004). The transported peptides are further degraded into eight to nine amino acid peptides by the ER-aminopeptidase I. The ER chaperones calnexin, calreticulin, and tapasin load the peptides onto MHC class I chains in the “MHC class I loading complex” to form the mature MHC class I molecules. The latter can then dissociate from the “loading complex” for transportation onto the surface of the cell, where they are stably expressed on the plasma membrane (Brode and Macary 2004; Heath et al. 2004; Théry and Amigorena 2001).

Although the description above explains the presentation of endogenous antigen such as that coming from intra-

cellular pathogens, it does not explain how DCs present antigens from tumor cells. It is clear that DCs can process and present both endogenous (generated within the DCs) and exogenous (endocytosed by the DC) antigens in the context of MHC class II. Similarly, MHC class I processing can utilize either endogenous or exogenous sources of antigen. The latter explains how DCs can present antigen from tumor cells, pathogen-infected cells, and even apoptotic cells to Tc lymphocytes. This presentation in the context of MHC class I is referred to as “cross-priming” (Melief 2003) or “cross-presentation” (Brode and Macary 2004; Heath et al. 2004). Indeed, cross-presentation has now been demonstrated to occur with DCs endocytosing virus antigens, particulate antigens, and proteins such as albumins, tumor cells, and apoptotic cells both in vitro and in vivo (reviewed by Brode and Macary 2004, Heath et al. 2004; Melief 2003).

Depending on the nature of the antigen or cell to be endocytosed, internalization by the DC uses one of several forms of endocytosis. In this context, it relates to uptake by DC for processing into the MHC class II presentation pathway. The difference is in the cytosolic targeting. With MHC class II processing, the target is the endosomal/lysosomal processing pathway that leads into the MIIC. For MHC class I processing to be activated, the endocytosed material must escape the lysosomal degradation by translocation into the cytosol. From here, the same process as for endogenous antigen processing occurs: ubiquitination, proteasome degradation, and transport into the ER (Brode and Macary 2004; Heath et al. 2004; Rodriguez et al. 1999).

Although DCs are important for cross-presentation, evidence has shown that other cells such as macrophages can also perform this function (Heath et al. 2004). Nevertheless, DCs are the most potent effectors of cross-presentation despite the fact that not all DCs perform this function equally. The DC family is a collection of subsets, and evidence has shown that the murine CD8 α ⁺CD205⁺ DC subset is the major subset involved in cross-presentation. Although this work must still be expanded into other species, it is clear that approaches to vaccine targeting of a particular aspect of immune defenses should include consideration of the subsets of leukocytes likely to be involved and therefore to be efficient for the immunization process.

DC Interaction with B Lymphocytes

DCs form clusters with B lymphocytes in vivo (Kushnir et al. 1998) and can be involved in their activation through antigen delivery (Litinskiy et al. 2002; Ludewig et al. 2000; Wykes et al. 1998). This interaction with B lymphocytes is related to “delivery” and is not a MHC-dependent “presentation” of antigen. It has also been reported that this DC-B cell interaction can result in stimulation of B cell differentiation into antibody-producing cells without the need for T cell help (Balazs et al. 2002; MacPherson et al. 1999). The antigen is unprocessed and has no MHC involvement. In addition, DCs play an important role in the regulation of humoral immunity through accessory molecule-dependent

regulation of B cell survival and proliferation. Examples include cognate interactions such as the CD40-dependent signaling, as well as secreted molecules such as the TNF-family ligands BAFF and APRIL (Mackay et al. 2003; MacLennan and Vinuesa 2002; Wykes and MacPherson 2000). The presentation of unprocessed antigen during DC-B cell interaction is not a consistent event, and it appears to be related to the antigen load delivered by the DC. The T cell-independent response is more readily identified under conditions of high antigen load. It is important to understand this factor when developing methods for the delivery of vaccines, and considering the antigen payload therein.

Stimulation of Specific Immune Responses

T Lymphocytes

The CD4⁺ Th lymphocytes are essential for efficient execution of the majority of specific immune responses, including antigen-activated B lymphocyte production of antibody (McHeyzer-Williams et al. 2000) and antigen-specific cytotoxic T lymphocytes (Tc) effector function (Mescher 1995). An important consideration is that not all Th lymphocytes recognize the same peptide; only distinctive Th lymphocyte clones recognize particular peptide sequences. This specificity is due to the antigen processing by the APCs, which results in several peptides that are capable of stimulating the Th lymphocytes for which they are specific (Blum et al. 1997; Van den Eynde and Morel 2001; Watts et al. 2003). These peptides carry what are referred to as T cell epitopes, which in general are continuous or sequential epitopes. Most show haplotype restriction, meaning that they will not be recognized by T lymphocytes from all individuals. Once stimulated, Th lymphocytes provide their “help” (hence the name T helper) via both cognate interactions and the release of soluble signaling mediators. The latter, cytokines, are responsible for driving antigen-stimulated B and Tc lymphocytes into becoming antibody-producing and effector cells, respectively. Of course, such cytokine signaling requires appropriate cognate interactions.

B Lymphocytes

Activation of B lymphocytes requires continued stimulation of both the membrane immunoglobulin-like receptors on the B lymphocytes by the antigen and the surface cytokine receptors by factors produced by the Th lymphocytes. In addition, a physical interaction between the B and Th lymphocytes is required. For example, CD40 and its ligand play a key role in permitting selective differentiation. Similar to the T lymphocyte responses, those of B lymphocytes are also restricted to particular clones that can recognize one of the epitopes on the antigen, referred to as the B cell epitopes. APCs are not required to process and present antigen to stimulate B lymphocytes, but it appears that they

play a role for the “delivery” of antigen into the B lymphocyte areas of the lymphoid follicles. This antigen must be “delivered” relatively intact because the epitopes recognized by B lymphocytes are most often discontinuous—they rely on the tertiary and quaternary (conformational) structure of the antigenic determinants on the antigen. Once a B lymphocyte interacts with the epitope for which it is specific, via the BCR, the cell is induced into capping its BCR in preparation for division and differentiation. At this point, the interaction with the Th lymphocytes, and in particular the involvement of the T cell cytokines, becomes critical. Without this interaction, the B lymphocyte reverts back to its resting stage, as before interaction with the antigen. In the presence of the correct cognate interaction with the Th lymphocytes and the involvement of the T cell cytokines, the B lymphocyte begins its differentiation pathway into the antibody-producing plasma cell. It is for this reason that a number of the T cell cytokines involved were originally referred to as B cell differentiation factors.

Innate-specific Interactions: Effector Immune Defenses

Interaction with the innate immune parts continues after induction of the specific immune defenses. At this point, the immune response is in a phase termed “the effector phase.” This term refers to the processes directly involved in effecting protection against the danger in question—pathogen, toxin, or other foreign substance considered by the immune system recognition processes as presenting a danger to the host. Interaction between the innate and specific immune parts can be seen with both antibody-based and cytotoxic effector immune defenses.

When antibody specific for the antigen in question binds to that antigen, the resultant immune complexes must be removed from the host. When the antigen is a pathogen, it is not possible to guarantee that the pathogen will not continue to infect its target cells (McCullough et al. 1992). Furthermore, immune complexes themselves can cause immunopathological disorders (e.g., Schifferli and Taylor 1989). MΦs are important innate defenses that interact with antibody-based effector defenses, and remove and degrade immune complexes (e.g., McCullough et al. 1986, 1988; Rigden et al. 2002). Without the presence of the antibody, the MΦs can phagocytose and destroyed the pathogen, although this action may be delayed and may even allow survival and transport of the pathogen in question (e.g., Rigden et al. 2002). Furthermore, many viruses and certain bacteria actually target MΦs and other cells of the innate immune system for infection and replication (e.g., Reiling et al. 2001; Suhrbier and La Linn 2003).

The cytotoxic immune defenses also utilize both specific (Tc lymphocytes) and innate (NK plus lymphokine-activated killer cells) processes. As mentioned above, the Tc lymphocytes are activated by MHC Class I-dependent recognition processes (Doherty et al. 1997). NK cells employ MHC-independent recognition, which permits the immune

defenses to identify, for example, virus-infected cells in which viral antigenic peptides are not presented on MHC molecules (see below). These NK cells will recognize what are referred to as “NK-specific, triggering surface molecules”—examples are NKp46, NKp30, and NKp44 (Moretta et al. 2001), but other triggering receptors are known (Biassoni et al. 2001). Another subset of T lymphocytes—the $\gamma\delta$ T cells—can recognize antigen without requiring MHC and therefore antigen processing (Boismenu and Havran 1997; Kabelitz et al. 2004). This knowledge has led to the proposal that such reactions of $\gamma\delta$ T cells may fulfill more of an innate than a specific defensive role. The innate and specific immune parts also interact in the regulation of cytotoxic defenses. NK cells produce IFN- γ and TGF- β , potent regulators of MΦs and lymphocytes, while MΦs and DCs can produce IL-12, which is capable of inducing NK cell and T lymphocyte production of the IFN- γ involved in growth regulation of these latter cells (Biron et al. 1999; Horwitz et al. 1999; Trinchieri 1995).

Cytotoxic immune defenses are important for destroying modified host cells such as tumor cells, as well as host cells infected with intracellular pathogens such as viruses (Kanavaros et al. 2000; Borrow 1997; Doherty et al. 1997). Tc lymphocytes and NK cells are major players in this context, recognizing antigenic peptides on an infected cell surface. Both of these groups of cytotoxic cells, as well as the cytotoxic $\gamma\delta$ T lymphocytes, will employ what are referred to as granule-associated cytotoxic proteins (GACPs¹) to lyse their targets—the tumor or virus-infected host cell (Kanavaros et al. 2000; Atkinson and Bleackley 1995). Examples of these GACPs are the T cell intracellular antigen-1, perforin, and granzyme B. In addition, there is evidence that Fas-Fas ligand interactions relating to induction of apoptosis may be implicated in the cytotoxic immune defenses (Doherty et al. 1997). When considering cytotoxic immune defenses, it is important to note that MΦs also play a role. MΦs can recognize antibody and complement components interacting with viral proteins expressed on the infected cell surface.

Taking all of these effector cytotoxic immune defenses together, it becomes evident that they are involved in destruction of infected cells only when the host cell surface is modified. Certain intracellular pathogens can evade detection, by not expressing antigenic proteins or peptides on the cell surface, or otherwise not modifying the cell surface, to avoid detection by the immune defenses (Borrow 1997; Borrow and Shaw 1998; Farrel and Davis-Poynter 1998). Nevertheless, the immune defenses can still recognize such infected cells if the infection results in cell death (apoptosis)—this is a capacity found with MΦs and other phagocytic cells of the immune system (Brode and Macary 2004; Heath et al. 2004).

Immunological Memory

When the threat posed by an antigen is overcome (i.e., when no more free antigen is stimulating the lymphocytes), the

immune system enters the phase of memory development (Crotty and Ahmed 2004; Gray 2002; Zinkernagel 2002). The important signal is the replacement of antigen by antigen/antibody complexes due to the progression of the immune system into antibody production. The low-affinity Fc receptor type II for immunoglobulin G (Fc γ RII¹) and Fc receptor type III for immunoglobulin G (Fc γ RIII¹) on phagocytes readily bind antibody that is complexed with antigen, rather than free antibody. Through such interactions, the cytoplasmic portion of the Fc γ RII and Fc γ RIII is modified, resulting in enhanced phagocytosis. This event is important in effector immune defense involving antibody and phagocytes.

An additional consequence of antibody/antigen complex formation is the interaction with B lymphocytes, in which case the low-affinity immunoglobulin receptor Fc γ RIB is involved. With B lymphocytes specific for the antigen, the antibody in the complexes interacts with the Fc γ RIB while the antigen in the complex interacts with the BCR. This cross-linking of the two receptors induces a different signaling within the B lymphocyte from that induced by antigen alone. The B cell differentiation switches from antibody-producing plasma cell to memory B lymphocyte development. Modifications of the surface interactions between B and Th lymphocytes and the cytokines involved are also implicated in the switch from antibody production to immunological memory development. In addition, the Th lymphocyte activity moves into the development of memory Th lymphocytes. Moreover, memory development concomitantly increases the number of antigen-reactive B and T lymphocytes, as well as the affinity of the BCR for the antigenic determinants for which they are specific. These modifications reflect the somatic mutation and selection involved with the increasing avidity of antibody and B lymphocyte receptor upon repeated exposure to the same or related antigens (Burnet 1959; Talmage 1957).

Cytokines

In addition to the cellular components, cytokines are important for the development of immune responses involved in the regulation of those responses (Hunter and Reiner 2000; Khaled and Durum 2002). The cytokines that are produced depend on the cells involved. For example, DCs can secrete IL-1, IL-6, IL-10, IL-12, TNF α , and Type I interferons; T lymphocytes produce IL-2, IL-4, IL-5, IL-10, TNF β , and IFN γ . Th lymphocyte cytokines can be described as “Th1” or “Th2” cytokines, due to the discovery in mice that individual Th lymphocyte clones secrete a particular pattern of cytokines. Th1 cytokines include IL-2, IFN γ , and TNF β . Th2 cytokines include IL-4, IL-5, IL-6, IL-10, and IL-13. Although such discrimination relates to the characteristics of an immune response, there is not the same subset relationship to Th1 and Th2 lymphocytes in humans that is seen in mice. The situation is further complicated by the fact that certain Th2 cytokines (e.g., IL-6 and IL-10) are also produced by monocytes and DCs.

Cytokines do not act in isolation. Rather, their effects are due to interactions with other cytokines. The result is a control over the development and characteristics of the immune response. It appears that this control is often designed to meet the threat imposed, such as responding when there is a danger to the host but regulating the response when the threat of danger has been controlled. Such measures ensure that the host does not respond against substances that should be tolerated (e.g., food), or continues responding in the absence of a threat. Such events can occur when the control procedures break down, leading to intolerance and even food allergy, or in the case of immunopathological disorders, when responses occur in the absence of a threat. Certain cytokines have been found to have particularly profound roles in development and control of immune responses. For example, interaction of Th lymphocytes with antigen-presenting DCs that are still in an immature state (iDCs) results in IL-10 production, which enhances regulatory T lymphocyte (Treg¹) activity and anergy of T cell responsiveness (Adorini 2003; Jonuleit et al. 2000). If the interaction involves mDC rather than iDC with Th lymphocytes, the latter produce IL-2 and IFN- γ , which promotes Th lymphocyte expansion. Importantly, when these Treg lymphocytes become involved, a shift occurs in cytokine expression from IFN- γ to IL-10, along with the downregulation of Th cell responses.

When dealing with immunization, one encounters the use of the term “Th1 cytokine response” (Jankovic et al. 2001; Maldonado-Lopez and Moser 2001). This term denotes that the immune activity involves cytokines that are characteristic of those produced by Th1 cells in mice. However, the term can be misleading with respect to vaccine efficacy. A Th1 cytokine profile is rarely induced in isolation from a Th2 cytokine profile. Certainly the cytokine profile may be dominated more by the Th1 cytokines than by the Th2 cytokines, but both groups are likely to be present. This characteristic is important for the regulation of the immune response, which relies on the balance between these groups.

Designing Vaccines and Immunization Strategy with the Aid of Immunological Profiles

Using Immunological Memory

In immunological memory, as mentioned above, the recall immune response in an immune host is more rapid and avid upon subsequent encounter with the antigen for which it is specific. In other words, the efficacy of immune defense is increased. It is this increased efficacy that is sought through any immunization, including vaccination—the aim of immunization in general is to induce a desired immune response, whereas vaccination is somewhat more targeted in preparing the host for defense against the pathogen or toxin in question. Clearly one must initiate the immune response

process with a naive host (i.e., begin with the primary immune response). With this method, the efficient targeting of DCs is critical (see below) due to their unique role in antigen presentation with primary immune response development. A second immunization will increase the efficiency of the induced immune defenses to respond against the antigen in question. Although other APCs, including B lymphocytes, can be involved at this point, the most efficient APC and therefore immunization target remains the DCs for stimulation of all parts of specific immune defense. The value of the secondary or booster immunizations arises from the fact that the populations of responding lymphocytes have been expanded, and their affinity for recognized epitopes has increased. In other words, both the rate and efficacy of antigen recognition and immune response are increased. With booster immunizations, these pools of high-affinity lymphocytes are further elaborated both in size and in the strength of epitope recognition. This elaboration leads to an increase in the efficiency with which the immune defenses can respond to subsequent encounter with the antigen, and indeed defend the host against that antigen when the goal is efficacious vaccination.

Promoting Appropriate Cytokine Profiles

The efficiency with which immune defenses can be stimulated by immunization (both effector immune defense and memory development) relies on the efficacy of the antigen or vaccine being employed. The result depends on how much is actually processed by the APCs and how much reaches the lymphoid follicles and germinal centers wherein the specific lymphocytes will be stimulated. Increasing the likelihood that the antigen or vaccine will be efficacious at inducing immune responses relies on appropriate targeting of the immune system (see below), as well as the application of adjuvants. Although adjuvants can assist targeting, the focus in this area has tended to be toward augmenting immune response development. In this latter context, certain cytokines can be applied as adjuvants. It should be remembered, however, that cytokines are growth factors and can be a double-edged sword—both stimulating immune responsiveness when that response is required and downregulating the responses when the threat to the host has been “neutralized.” Application of cytokines as adjuvants can be advantageous, certainly when initiating immune responses, but such cytokine application should be considered carefully. It is essential to ensure that the correct form of interaction with the immune system is being effected.

An alternative to cytokines as adjuvants is to use biologically defined adjuvants that induce a known cytokine profile. In this area, lipoproteins, lipopeptides, CpG-ODN motifs, and antigenic structures such as Bappamun are particularly potent, and have had some success. It is important to characterize and have a detailed understanding of the cytokine profiles induced by particular adjuvants. For example, application of aluminium hydroxide as an adjuvant

can promote primarily a Th2-like response (Brewer et al. 1999). However, the type of response induced actually depends on the antigen in question, and the influence of whether a primary or secondary immune response is being activated (Wilcock et al. 2004).

It is also important to understand adjuvant-induced cytokine profiles because of the reason for their application—to enhance induction of efficacious immune defenses. It is important to exercise care when applying anything with immunomodulatory capacity, because the immune responses will be modified depending on the cytokine profiles induced (e.g., see reviews by Krieg 2002; Partidos et al. 2004). Furthermore, immune defenses against particular pathogens will benefit from a profile dominated by Th1- or Th2-like cytokines depending on the pathogen (e.g., see reviews by Jankovic et al. 2001; von Stebut and Udey 2004). Adjuvants can also influence in an immunological targeting sense, because particular DC subsets are involved in the regulation of Th1 and Th2 cytokine responses (Maldonado-Lopez and Moser 2001). Clearly it is important to understand the characteristics of an efficacious defense against the pathogen or antigen in question. Only with this knowledge can one determine the appropriate components to be employed in an immunization or vaccination.

Immunological Target for Vaccination

Induction of long-lasting protective immunity is the primary aim in vaccination against infectious diseases or toxins. As mentioned above, the pivotal cell in the complex immune network is the DC. This distinct family of white blood cells belongs to the first line of immune defense against pathogen attack. Due to their migration throughout the body (including the subepithelial areas at mucosal surfaces) as they seek out anything presenting a danger for the host, they capture invading pathogens or the administered vaccine. The DCs can carry this material into the lymphoid follicles and germinal centers for delivery to B lymphocytes and presentation of the processed antigen to T lymphocytes. By this method, protective immune responses—the desired end-product of vaccination—are stimulated. Because of the critical nature of the DC activity for the efficiency of the immune response induction, the design must take into account both the lymphocytes’ recognition of antigen specificity within the vaccine and the need for efficient DC activation. As described above, when DCs capture pathogen or vaccine antigen, they are in an ‘immature’ state. For efficient delivery and processing of that antigen, the DCs must respond to what is described as a danger signal, which is responsible for inducing their maturation. Therefore, vaccines must target DCs as well as induce their maturation. Only with both processes can one be assured that the DCs will efficiently stimulate lymphocytes into an active immune defense response. Without DC maturation, the induced response will be ineffective or may even result in a state of tolerance (see above).

Numerous methods for targeting DC have been reported, including ligands for receptors on the DC surface (Diefenbach and Raulet 2003; Gordon 2002; Vasselon and Detmers 2002). Of particular interest are the TLRs, which can be used not only to target the DC but also for interaction, which activates the DC and often results in maturation. A good example of these activities is seen with adjuvants containing lipoprotein or lipopeptide moieties. These adjuvants target TLR2 and/or TLR4, and stimulate DC maturation (Weigt et al. 2003). It is not always necessary for the receptors to be on the cell surface. For example, although TLR9 is internal, it is the receptor for CpG-ODN (Bauer and Wagner 2002; Sparwasser and Lipford 2000). Actually, CpG-ODNs do not react with all DCs, but only with the subset known as plasmacytoid DCs, or natural interferon-producing cells (NIPCs¹) (Rothenfusser et al. 2002). This result is a clear contrast between the murine and human immune systems: CpG-ODNs stimulate all DCs in murine cells, but only the NIPCs in human (Hochrein et al. 2002). In addition, with porcine DCs, only NIPCs respond to CpG-ODNs, making this animal model more appropriate for adjuvant studies using CpG-ODNs (Guzylack-Piriou et al. 2004; Hochrein and Wagner 2004).

CpG-ODN stimulation of NIPCs is particularly important due to the high levels of IFN- α along with TNF- α that are induced (Rothenfusser et al. 2002). IFN- α and TNF- α are important stimulators of DC maturation (Le Bon and Tough 2002; Luft et al. 1998) and NK cell activation (Brasard et al. 2002), and they enhance humoral immunity (Le Bon et al. 2001). This is one reason for the success of CpG-ODN application as adjuvants; however, the reasoning is more complex because only type A CpG-ODNs stimulate NIPCs. Another type, type B, is reported to stimulate B lymphocytes directly, whereas type C can stimulate both NIPC and B lymphocytes (Klinman 2004). The direct stimulation of B cells by type B or C CpG-ODN not only induces a polyclonal activation with proliferation but also increases production of IL-6 and chemokines as well as increasing antibody secretion. It is evident that these potent effects of CpG also have the potential to cause damage such as autoimmune diseases (Klinman 2004).

Conclusions

The precise interaction of monocytes, DCs, and T and B lymphocytes during inductive phases is centrally important to immune response development. These cells control cytokine production and antigen delivery at the exact time and place required, as well as providing the correct levels of stimulation and control for correct immune response development. Effective vaccine formulations are superior to administration of the cytokines themselves because the vaccine and its formulation components will stimulate the immune system to produce the responses that include the required cytokines. Also critical in the development of the immune response is the delivery of intact virus anti-

gen to the B lymphocytes and of processed antigen (peptide in association with MHC class II molecules) to the Th lymphocytes. It is essential not only to recognize and understand the different, important roles of antibody-based and cytotoxic immune defenses but also to match the requirements for protection against the pathogen or toxin in question with the development of the protective immune responses.

References

- Adorini L. 2003. Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting autoimmune diabetes. *Ann N Y Acad Sci* 987:258-261.
- Atkinson EA, Bleackley RC. 1995. Mechanisms of lysis by cytotoxic T cells. *Crit Rev Immunol* 15:359-384.
- Balazs M, Martin F, Zhou T, Kearney J. 2002. Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity* 17:341-352.
- Bals R. 2000. Epithelial antimicrobial peptides in host defense against infection. *Respir Res* 1:141-150.
- Banchereau J, Steinman RM. 1998. Dendritic cells and the control of immunity. *Nature* 392:245-252.
- Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. 2000. Immunobiology of dendritic cells. *Annu Rev Immunol* 18:767-811.
- Barrington R, Zhang M, Fischer M, Carroll MC. 2001. The role of complement in inflammation and adaptive immunity. *Immunol Rev* 180:5-15.
- Basta S, Carrasco CP, Knoetig SM, Rigden RC, Gerber H, Summerfield A, McCullough KC. 2000. Porcine alveolar macrophages: Poor accessory or effective suppressor cells for T-lymphocytes. *Vet Immunol Immunopathol* 77:177-190.
- Bauer S, Wagner H. 2002. Bacterial CpG-DNA licenses TLR9. *Curr Top Microbiol Immunol* 270:145-154.
- Beutler B. 2000. Tlr4: Central component of the sole mammalian LPS sensor. *Curr Opin Immunol* 12:20-26.
- Biassoni R, Cantoni C, Pende D, Sivori S, Parolini S, Vitale M, Bottino C, Moretta A. 2001. Human natural killer cell receptors and co-receptors. *Immunol Rev* 181:203-214.
- Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. 1999. Natural killer cells in antiviral defense: Function and regulation by innate cytokines. *Annu Rev Immunol* 17:189-220.
- Blum JS, Ma C, Kovats S. 1997. Antigen-presenting cells and the selection of immunodominant epitopes. *Crit Rev Immunol* 17:411-417.
- Boismenu R, Havran WL. 1997. An innate view of gamma delta T cells. *Curr Opin Immunol* 9:57-63.
- Borrow P. 1997. Mechanisms of viral clearance and persistence. *J Viral Hepat* 4(Suppl 2):16-24.
- Borrow P, Shaw GM. 1998. Cytotoxic T-lymphocyte escape viral variants: How important are they in viral evasion of immune clearance in vivo? *Immunol Rev* 164:37-51.
- Bozza S, Gaziano R, Spreca A, Bacci A, Montagnoli C, di Francesco P, Romani L. 2002. Dendritic cells transport conidia and hyphae of *Aspergillus fumigatus* from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. *J Immunol* 168:1362-1371.
- Brassard DL, Grace MJ, Bordens RW. 2002. Interferon-alpha as an immunotherapeutic protein. *J Leuk Biol* 71:565-581.
- Brewer JM, Conacher M, Hunter CA, Mohrs M, Brombacher F, Alexander J. 1999. Aluminium hydroxide adjuvant initiates strong antigen-specific Th2 responses in the absence of IL-4- or IL-13-mediated signaling. *J Immunol* 163:6448-6454.
- Brode S, Macary PA. 2004. Cross-presentation: Dendritic cells and macrophages bite off more than they can chew! *Immunology* 112:345-351.

- Burnet FM. 1959. *The Clonal Selection Theory of Acquired Immunity*. Cambridge: Cambridge University Press.
- Crotty S, Ahmed R. 2004. Immunological memory in humans. *Semin Immunol* 16:197-203.
- Diefenbach A, Raulet DH. 2003. Innate immune recognition by stimulatory immunoreceptors. *Curr Opin Immunol* 15:37-44.
- Doherty PC, Topham DJ, Tripp RA, Cardin RD, Brooks JW, Stevenson PG. 1997. Effector CD4⁺ and CD8⁺ T-cell mechanisms in the control of respiratory virus infections. *Immunol Rev* 159:105-117.
- Farrell HE, Davis-Poynter NJ. 1998. From sabotage to camouflage: Viral evasion of cytotoxic T lymphocyte and natural killer cell-mediated immunity. *Semin Cell Dev Biol* 9:369-378.
- Feger F, Varadaradjalou S, Gao Z, Abraham SN, Arock M. 2002. The role of mast cells in host defense and their subversion by bacterial pathogens. *Trends Immunol* 23:151-158.
- Fellermann K, Stange EF. 2001. Defensins—Innate immunity at the epithelial frontier. *Eur J Gastroenterol Hepatol* 13:771-776.
- Finkelman FD, Lees A, Morris SC. 1992. Antigen presentation by B lymphocytes to CD4⁺ T lymphocytes in vivo: Importance for B lymphocyte and T lymphocyte activation. *Semin Immunol* 4:247-255.
- Fraser IP, Koziel H, Ezekowitz RA. 1998. The serum mannose-binding protein and the macrophage mannose receptor are pattern recognition molecules that link innate and adaptive immunity. *Semin Immunol* 10:363-372.
- Gallucci S, Matzinger P. 2001. Danger signals: SOS to the immune system. *Curr Opin Immunol* 13:114-119.
- Gasque P. 2004. Complement: A unique innate immune sensor for danger signals. *Mol Immunol* 41:1089-1098.
- Gatti E, Pierre P. 2003. Understanding the cell biology of antigen presentation: The dendritic cell contribution. *Curr Opin Cell Biol* 15:468-473.
- Gordon S. 2002. Pattern recognition receptors: Doubling up for the innate immune response. *Cell* 111:927-930.
- Gray D. 2002. A role for antigen in the maintenance of immunological memory. *Nat Rev Immunol* 2:60-65.
- Guermontprez P, Valladeau J, Zitvogel L, Théry C, Amigorena S. 2002. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* 20:621-667.
- Gunn MD. 2003. Chemokine mediated control of dendritic cell migration and function. *Semin Immunol* 15:271-276.
- Guzylack-Piriou L, Balmelli C, McCullough KC, Summerfield A. 2004. Type-A CpG oligonucleotides activate exclusively porcine natural interferon-producing cells to secrete interferon-alpha, tumour necrosis factor-alpha and interleukin-12. *Immunology* 112:28-37.
- Heath, W.R., Belz, G.T., Behrens, G.M., Smith, C.M., Forehan, S.P., Parish, I.A., Davey, G.M., Wilson, N.S., Carbone, F.R., Villadangos, J.A. 2004. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol Rev* 199:9-26.
- Hochrein, H., O'Keeffe, M., Wagner, H. 2002. Human and mouse plasmacytoid dendritic cells. *Hum Immunol* 63:1103-1110.
- Hochrein, H., Wagner, H. 2004. Of men, mice and pigs: Looking at their plasmacytoid dendritic cells. *Immunology* 112:26-27.
- Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. 1999. Phylogenetic perspectives in innate immunity. *Science* 284:1313-1318.
- Holt PG, Stumbles PA, McWilliam AS. 1999. Functional studies on dendritic cells in the respiratory tract and related mucosal tissues. *J Leuk Biol* 66:272-275.
- Horwitz DA, Gray JD, Ohtsuka K. 1999. Role of NK cells and TGF-beta in the regulation of T-cell-dependent antibody production in health and autoimmune disease. *Microbes Infect* 1:1305-1311.
- Hunter CA, Reiner SL. 2000. Cytokines and T cells in host defense. *Curr Opin Immunol* 12:413-418.
- Janeway CA Jr, Medzhitov R. 2002. Innate immune recognition. *Annu Rev Immunol* 20:197-216.
- Jankovic D, Liu Z, Gause WC. 2001. Th1- and Th2-cell commitment during infectious disease: Asymmetry in divergent pathways. *Trends Immunol* 22:450-457.
- Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH. 2000. Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 192:1213-1222.
- Kabelitz D, Wesch D, Pitters E, Zoller M. 2004. Potential of human gamma-delta T lymphocytes for immunotherapy of cancer. *Int J Cancer* 112:727-732.
- Kanavaros P, Boulland ML, Petit B, Arnulf B, Gaulard P. 2000. Expression of cytotoxic proteins in peripheral T-cell and natural killer-cell (NK) lymphomas: Association with extranodal site, NK or Tgammadelta phenotype, anaplastic morphology and CD30 expression. *Leuk Lymphoma* 38:317-326.
- Khaled AR, Durum SK. 2002. Lymphocyte: Cytokines and the control of lymphoid homeostasis. *Nat Rev Immunol* 2:817-830.
- Klinman DM. 2004. Immunotherapeutic uses of CpG oligodeoxynucleotides. *Nat Rev Immunol* 4:249-258.
- Knight SC, Stagg AJ. 1993. Antigen-presenting cell types. *Curr Opin Immunol* 5:374-382.
- Krieg AM. 2002. CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol* 20:709-760.
- Kushnir N, Liu L, MacPherson GG. 1998. Dendritic cells and resting B cells form clusters in vitro and in vivo: T cell independence, partial LFA-1 dependence, and regulation by cross-linking surface molecules. *J Immunol* 160:1774-1781.
- Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. 2001. Type I interferons potentially enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. *Immunity* 14:461-470.
- Le Bon A, Tough DF. 2002. Links between innate and adaptive immunity via type I interferon. *Curr Opin Immunol* 14:432-436.
- Lelouard H, Ferrand V, Marguet D, Bania J, Camosseto V, David A, Gatti E, Pierre P. 2004. Dendritic cell aggresome-like induced structures are dedicated areas for ubiquitination and storage of newly synthesized defective proteins. *J Cell Biol* 164:667-675.
- Lelouard H, Gatti E, Cappello F, Gresser O, Camosseto V, Pierre P. 2002. Transient aggregation of ubiquitinated proteins during dendritic cell maturation. *Nature* 417:177-182.
- Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, Cerutti A. 2002. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat Immunol* 3:822-829.
- Ludewig B, Maloy KJ, Lopez-Macias C, Odermatt B, Hengartner H, Zinkernagel RM. 2000. Induction of optimal anti-viral neutralizing B cell responses by dendritic cells requires transport and release of virus particles in secondary lymphoid organs. *Eur J Immunol* 30:185-196.
- Luft T, Pang KC, Thomas E, Hertzog P, Hart DN, Trapani J, Cebon J. 1998. Type I IFNs enhance the terminal differentiation of dendritic cells. *J Immunol* 161:1947-1953.
- Mackay CR. 2001. Chemokines: Immunology's high impact factors. *Nat Immunol* 2:95-101.
- Mackay F, Schneider P, Rennert P, Browning J. 2003. BAFF AND APRIL: A tutorial on B cell survival. *Annu Rev Immunol* 21:231-264.
- MacLennan I, Vinuesa C. 2002. Dendritic cells, BAFF, and APRIL: Innate players in adaptive antibody responses. *Immunity* 17:235-238.
- MacPherson G, Kushnir N, Wykes M. 1999. Dendritic cells, B cells and the regulation of antibody synthesis. *Immunol Rev* 172:325-334.
- MacPherson GG, Liu LM. 1999. Dendritic cells and Langerhans cells in the uptake of mucosal antigens. *Curr Top Microbiol Immunol* 236:33-53.
- Maldonado-Lopez R, Moser M. 2001. Dendritic cell subsets and the regulation of Th1/Th2 responses. *Semin Immunol* 13:275-282.
- Matsushita M, Endo Y, Nonaka M, Fujita T. 1998. Complement-related serine proteases in tunicates and vertebrates. *Curr Opin Immunol* 10:29-35.
- McCullough KC, Crowther JR, Butcher RN, Carpenter WC, Brocchi E, Capucci L, De Simone F. 1986. Immune protection against foot-and-mouth disease virus studied using virus-neutralizing and non-neutralizing concentrations of monoclonal antibodies. *Immunology* 58:421-428.
- McCullough KC, De Simone F, Brocchi E, Capucci L, Crowther JR, Kihm

- U. 1992. Protective immune response against foot-and-mouth disease. *J Virol* 66:1835-1840.
- McCullough KC, Parkinson D, Crowther JR. 1988. Opsonization-enhanced phagocytosis of foot-and-mouth disease virus. *Immunology* 65:187-191.
- McHeyzer-Williams MG, McHeyzer-Williams LJ, Fanelli Panus J, Bikah G, Pogue-Caley RR, Driver DJ, Eisenbraun MD. 2000. Antigen-specific immunity: Th cell-dependent B cell responses. *Immunol Res* 22:223-236.
- Medzhitov R, Janeway C Jr. 2000a. Innate immune recognition: Mechanisms and pathways. *Immunol Rev* 173:89-97.
- Medzhitov R, Janeway C Jr. 2000b. The Toll receptor family and microbial recognition. *Trends Microbiol* 8:452-456.
- Medzhitov R, Janeway CA Jr. 1999. Innate immune induction of the adaptive immune response. *Cold Spring Harb Symp Quant Biol* 64:429-435.
- Melief CJ. 2003. Mini-review: Regulation of cytotoxic T lymphocyte responses by dendritic cells: Peaceful coexistence of cross-priming and direct priming? *Eur J Immunol* 33:2645-2654.
- Mellman I, Steinman RM. 2001. Dendritic cells: Specialized and regulated antigen processing machines. *Cell* 106:255-258.
- Mescher MF. 1995. Molecular interactions in the activation of effector and precursor cytotoxic T lymphocytes. *Immunol Rev* 146:177-210.
- Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, Biassoni R, Moretta L. 2001. Activating receptors and coreceptors involved in human natural killer cell-mediated cytotoxicity. *Annu Rev Immunol* 19:197-223.
- Murdoch C, Finn A. 2000. Chemokine receptors and their role in inflammation and infectious diseases. *Blood* 95:3032-3043.
- Netea MG, van der Graaf C, Van der Meer JW, Kullberg BJ. 2004. Toll-like receptors and the host defense against microbial pathogens: Bringing specificity to the innate-immune system. *J Leuk Biol* 75:749-755.
- Partidos CD, Beignon AS, Briand JP, Muller S. 2004. Modulation of immune responses with transcutaneously deliverable adjuvants. *Vaccine* 22:2385-2390.
- Pulendran B, Palucka K, Banchereau J. 2001. Sensing pathogens and tuning immune responses. *Science* 293:253-256.
- Randolph GJ. 2001. Dendritic cell migration to lymph nodes: Cytokines, chemokines, and lipid mediators. *Semin Immunol* 13:267-274.
- Reiling N, Klug K, Krallmann-Wenzel U, Laves R, Goyert S, Taylor ME, Lindhorst TK, Ehlers S. 2001. Complex encounters at the macrophage-mycobacterium interface: Studies on the role of the mannose receptor and CD14 in experimental infection models with *Mycobacterium avium*. *Immunobiology* 204:558-571.
- Rescigno M, Rotta G, Valzasina B, Ricciardi-Castagnoli P. 2001a. Dendritic cells shuttle microbes across gut epithelial monolayers. *Immunobiology* 204:572-581.
- Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, Granucci F, Kraehenbuhl JP, Ricciardi-Castagnoli P. 2001b. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2:361-367.
- Rigden RC, Carrasco CP, Summerfield A, McCullough KC. 2002. Macrophage phagocytosis of foot-and-mouth disease virus may create infectious carriers. *Immunology* 106:537-548.
- Rodriguez A, Regnault A, Kleijmeer M, Ricciardi-Castagnoli P, Amigorena S. 1999. Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. *Nat Cell Biol* 1:362-368.
- Romani N, Koide S, Crowley M, Witmer-Pack M, Livingstone AM, Fathman CG, Inaba K, Steinman RM. 1989. Presentation of exogenous protein antigens by dendritic cells to T cell clones: Intact protein is presented best by immature, epidermal Langerhans cells. *J Exp Med* 169:1169-1178.
- Rossi D, Zlotnik A. 2000. The biology of chemokines and their receptors. *Annu Rev Immunol* 18:217-242.
- Rothenfusser S, Tuma E, Endres S, Hartmann G. 2002. Plasmacytoid dendritic cells: The key to CpG. *Hum Immunol* 63:1111-1119.
- Schifferli JA, Taylor RP. 1989. Physiological and pathological aspects of circulating immune complexes. *Kidney Int* 35:993-1003.
- Schuler G, Steinman RM. 1985. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. *J Exp Med* 161:526-546.
- Shi Y, Evans JE, Rock KL. 2003. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 425:516-521.
- Sparwasser T, Lipford GB. 2000. Consequences of bacterial CpG DNA-driven activation of antigen-presenting cells. *Curr Top Microbiol Immunol* 247:59-75.
- Stahl PD, Ezekowitz RA. 1998. The mannose receptor is a pattern recognition receptor involved in host defense. *Curr Opin Immunol* 10:50-55.
- Steinman RM. 1991. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 9:271-296.
- Steinman RM, Inaba K, Turley S, Pierre P, Mellman I. 1999. Antigen capture, processing, and presentation by dendritic cells: Recent cell biological studies. *Hum Immunol* 60:562-567.
- Suhrbier A, La Linn M. 2003. Suppression of antiviral responses by antibody-dependent enhancement of macrophage infection. *Trends Immunol* 24:165-168.
- Summerfield A, Guzylack-Piriou L, Schaub A, Carrasco CP, Horn MP, Tâche V, Charley B, McCullough KC. 2003. Porcine peripheral blood dendritic cells and natural interferon producing cells. *Immunology* 110:1-10.
- Takeda K, Akira S. 2001. Roles of Toll-like receptors in innate immune responses. *Genes Cells* 6:733-742.
- Talmage DW. 1957. Diversity of antibodies. *J Cell Physiol* 50(Suppl 1):229-246.
- Théry C, Amigorena S. 2001. The cell biology of antigen presentation in dendritic cells. *Curr Opin Immunol* 13:45-51.
- Triantafilou M, Triantafilou K. 2002. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends Immunol* 23:301-304.
- Trinchieri G. 1995. Interleukin-12: A proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Rev Immunol* 13:251-276.
- Van den Eynde BJ, Morel S. 2001. Differential processing of class-I-restricted epitopes by the standard proteasome and the immunoproteasome. *Curr Opin Immunol* 13:147-153.
- Vasselon T, Detmers PA. 2002. Toll receptors: A central element in innate immune responses. *Infect Immun* 70:1033-1041.
- von Stebut E, Udey MC. 2004. Requirements for Th1-dependent immunity against infection with *Leishmania major*. *Microbes Infect* 6:1102-1109.
- Watts C, Moss CX, Mazzeo D, West MA, Matthews SP, Li DN, Manoury B. 2003. Creation versus destruction of T cell epitopes in the class II MHC pathway. *Ann N Y Acad Sci* 987:9-14.
- Weigt H, Muhlradt PF, Emmendorffer A, Krug N, Braun A. 2003. Synthetic mycoplasma-derived lipopeptide MALP-2 induces maturation and function of dendritic cells. *Immunobiology* 207:223-233.
- Wilcock LK, Francis JN, Durham SR. 2004. Aluminium hydroxide down-regulates T helper 2 responses by allergen-stimulated human peripheral blood mononuclear cells. *Clin Exp Allergy* 34:1373-1378.
- Wykes M, MacPherson G. 2000. Dendritic cell-B-cell interaction: Dendritic cells provide B cells with CD40-independent proliferation signals and CD40-dependent survival signals. *Immunology* 100:1-3.
- Wykes M, Pombo A, Jenkins C, MacPherson GG. 1998. Dendritic cells interact directly with naive B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response. *J Immunol* 161:1313-1319.
- Zinkernagel RM. 2002. On differences between immunity and immunological memory. *Curr Opin Immunol* 14:523-536.
- Zinkernagel RM, Doherty PC. 1997. The discovery of MHC restriction. *Immunol Today* 18:14-17.
- Zinkernagel RM, Doherty PC. 1979. MHC-restricted cytotoxic T cells: Studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function, and responsiveness. *Adv Immunol* 27:51-177.