Preface

The HIV epidemic has brought renewed attention to the immune system and an enhanced understanding of its mechanisms for defending against infection. Despite the development of potent chemotherapeutic agents against HIV, chronic HIV infection cannot be cured over the long term with this approach. Chronic exposure to these medications is limited by debilitating toxicities and the development of drug resistance. Hence, there is a need to understand how the immune system can be manipulated to effect better control of viral replication and disease progression. This effort is proceeding in tandem with progress toward development of an effective vaccine.

Other infections, particularly those for which the development of safe, effective chemotherapy has proved difficult, have been targeted with specific immunotherapeutic approaches, from monoclonal antibodies to vaccines to interferons and cytokines.

Immunotherapy for Infectious Diseases is intended to review the state-of-the-art developments of this rapidly emerging and evolving field. Much of the work in this area is only beginning to be appreciated by clinicians and medical scientists. We hope *Immunotherapy for Infectious Diseases* will not only serve as a useful guide to current knowledge of the field, but will also stimulate readers to contribute to its further development. As such, the book should be of interest to basic scientists and clinicians active in the fields of immunology and infectious diseases, particularly HIV infection.

Immunotherapy for Infectious Diseases is divided into four sections. The first section provides an overview of the basic principles of immune defense, as seen in the context of developing strategies of immunotherapy. Humoral and cellular immunity are reviewed. Because many infectious agents enter and exit through mucosal surfaces, there has been growing appreciation of the role of mucosal immunity in protection against infection and immunopathogenesis. Therefore, a chapter on mucosal immunity is included.

The second section discusses the principles of immunotherapy on a molecular level. There are discussions of monoclonal antibodies, types of vaccines, methods of antigen presentation, cytokines, and cytokine antagonists.

The third section reviews the current state of anti-HIV immunotherapy. The current knowledge of HIV immunopathogenesis is reviewed, as is the degree of immune reconstitution that occurs as a result of anti-HIV chemotherapy. Chapters dealing with HIV-specific passive and active immunization strategies, gene therapy, and host cell-targeted approaches for treating HIV infection and restoring immune function are presented.

The fourth section reviews immunotherapy for additional infections and virus-associated malignancies.

I am grateful to all of our experts who contributed chapters to the book. They represent some of the finest minds working in this area, and did superb jobs in reviewing the latest information in their areas of expertise. I am deeply appreciative of Dr. Vassil St. Georgiev, the series editor, for inviting me to edit this book, and Thomas Lanigan, Sr., Elyse O'Grady, Craig Adams and Diana Mezzina, at Humana Press for their support in compiling it. Thanks also to the secretaries and copy editors who diligently worked to put together the elements of the book. Finally, I wish to thank the readers, who I hope will use the knowledge gained from this book to advance our ability to treat infectious diseases.

Jeffrey M. Jacobson, MD

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Some Basic Cellular Immunology Principles Applied to the Pathogenesis of Infectious Diseases

R. Pat Bucy and Paul Goepfert

INTRODUCTION

In this chapter some of the functional implications of our current understanding of the basic physiology of T-cell mediated immune function for problems in infectious disease are discussed. The subtleties of the process of T-cell antigen "recognition" and the heterogeneity of kinds of functional responses within the T-cell system are a major focus. Finally, some features of the anatomic compartmentalization of the immune system and how limited access to tissue compartments skews our thinking about in vivo immunity in humans are explored. In view of our recently enhanced understanding of HIV disease, the chapter uses this viral infection as an example to illustrate relevant immune mechanisms and concepts.

MECHANISMS OF IMMUNE RECOGNITION

As outlined above, T-cells utilize a complex process to discriminate particular antigenic epitopes. Unlike antibodies that can bind with high affinity to multiple kinds of biomolecules, T-cells only recognize peptide epitopes that are embedded into one of two classes of specialized antigen-presenting structures (Fig. 1). The molecules were originally defined as strong transplantation antigens, coded for by a complex of genes termed the major histocompatibility complex (MHC). The class I MHC molecule exists on the surface of most nucleated cells, albeit at varying densities, in a complex with a small, constant component known as β_2-microglobulin. These molecules bind a selected set of peptides that are primarily derived from cytosolic proteins via degradation and transport into specialized membrane compartments by the proteosome transporter protein (TAP) complex. The peptide/class I molecules are expressed on the cell surface and serve as the antigenic stimulus for CD8+ T-cells. The CD8 molecule on the T-cell binds directly to framework portions of the class I MHC molecule, distinct from the peptide binding site and stabilizes the interaction of the T-cell receptor (TCR)/peptide/MHC complex. The class II MHC molecules serve a similar function of peptide binding and presentation, but they differ in several important ways. First, only selected cell types express class II molecules constitutively, although some cytokines (particularly interferon-γ [IFN-γ] and tumor necrosis factor-α [TNF-α]) can stimulate

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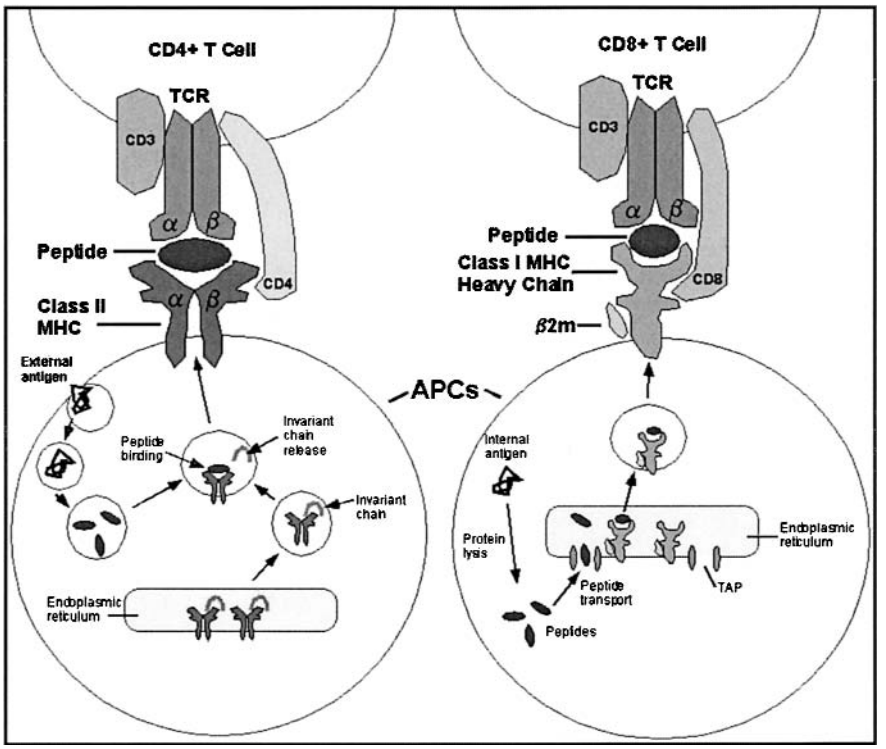

Fig. 1. Two pathways of antigen presentation correlating with two subsets of responding T cells. APCs, antigen-presenting cells; β2m, β_2-microglobulin; TCR, T-cell receptor.

other cells to express these molecules. Second, the class II molecule is a heterodimer of two different MHC-derived proteins with the peptide binding pocket having open ends allowing somewhat more flexibility in the selected peptides. Third, peptides derived from extracellular materials engulfed by the antigen-presenting cells (APCs) are loaded into class II molecules in distinct membrane-bound compartments, compared with the loading of class I molecules. Finally, CD4 binds to the framework portions of the class II MHC molecule to stabilize antigen recognition of CD4+ T-cells.

Thus, the two major sublineages of T-cells (CD4 and CD8) recognize antigens in two distinct kinds of presenting molecules that bind largely distinct universes of peptide determinants (intracellular vs extracellular), usually on distinct kinds of APCs. The binding affinity of the TCR with the MHC/peptide complex (K_d of 10^{-4}–10^{-7}M) is significantly less than typical antibody binding affinities. Several accessory membrane molecules are therefore required to increase this binding affinity. Some of the most notable of the accessory molecules include CD4 and CD8, which function by binding to specific domains of the MHC class II and class I molecules, respectively. Both the CD4 and CD8 molecules also act as signal tranducers, playing a role in intracellular signaling events. Other accessory proteins also play important roles in the TCR/MHC complex interaction, such as CD28, CD2, leukocyte function-associated antigen-(LFA-1), and CD45R *(1–8)*.

This intimate role of the MHC antigens in the process of T-cell recognition not only controls the induction of specific immune effector mechanisms, but is also critical for selection and maintenance of the repertoire of TCR specificities in the T-cell pool. Dur-

ing thymic development, randomly arranged TCR structures are tested out for low avidity to the available peptide/MHC molecules, presumably using peptides derived from ubiquitous self-components. Most TCR structures fail the twin selective processes of thymic repertoire selection: they either bind too strongly to available peptide/MHC molecules (functionally defined as self-antigen), or they fail to bind well enough to receive a positive survival signal *(9–11)*. In both of these situations, the T-cell is deleted, and the surviving T-cells have a low to intermediate binding affinity to a self-peptide/MHC molecular complex. The same type of low-avidity interactions with available peptide/MHC molecules also appears to be necessary for long-term survival of peripheral T-cells. Individual T-cells in the peripheral pool can undergo mitosis without developing the changes associated with specific memory function *(12,13)*, probably with one daughter cell undergoing apoptosis and the other surviving. Data from experiments using mice have shown that maintenance of the population depends on low-level TCR-mediated signals *(14–20)*.

Although most of the specific recognition characteristics inherent in the trimolecular complex mode of antigen recognition is mediated by the TCR repertoire generated during fetal development, each individual MHC molecule can only bind a fairly limited set of peptides with constrained structural features. Although this strategy apparently offers a degree of fine physiologic control (to prevent autoimmunity?), this mechanism results in alterations in the intensity of the immune response in different individuals with structurally different MHC molecules. Especially in immune responses to antigens of limited structural heterogeneity, the intensity of the response is often controlled by a genetic element linked to the MHC known as an immune response (Ir) gene. It is now clear that the structural gene that maps to the MHC is either the class I or class II antigen-presenting molecule; however, the "Ir gene phenotype" is a complex mixture of the structure of the TCR repertoire and the determinant selection activity of particular MHC molecules *(21,22)*. In some cases, there is a hole in the TCR repertoire such that a particular peptide MHC complex fails to stimulate any available T-cells; in other cases, antigenic peptides simply fail to bind with any of the available MHC restriction elements. In either case, the immune response to such an antigen is unproductive and this phenotype is a heritable genetic trait, an Ir gene.

This variability of immune response intensity due to structural constraints on permissive peptide binding by individual MHC molecules is thought to be related to the extreme polymorphism of these molecules maintained among individuals within the population. Not only are there several distinct loci for both class I and class II antigens (three for each class in humans), but there are multiple polymorphic alleles present at each locus. Since any one MHC molecule can present only a fairly limited repertoire of peptides, it is widely accepted that there is a significant selective advantage for a population to maintain great diversity of immune recognition structures. Such a diverse set of restriction elements serves to mitigate the likelihood of a single epidemic pathogen escaping detection by most individuals in a localized population.

The complex patterns of disease associations with particular alleles of the MHC, many of which involve the predisposition to autoimmune mechanisms, undoubtedly arise out of this central role of the trimolecular complex in the life, functional activity, and death of T-cells. Variation in response intensity with different MHC haplotypes can also lead to significant mechanistic insights. For example, the strong associations of

rates of HIV-1 disease progression in particular MHC class I alleles *(23–25)* suggest several strong implications about the role of cellular immunity in HIV disease. First, the statistical association of MHC molecules with disease progression rates correlates with the primary effect of a relationship of MHC molecules to the initial viral load set point *(24)*, which, in turn, determines the rate of disease progression. Second, the association of particular class I alleles and the advantageous effect of heterozygosity are the molecular signature of the critical role of CD8 T-cell antigen recognition in control of the viral load set point. Furthermore, the concept that CD8 T-cells play a central role in control of HIV infection is supported by a number of other independent lines of evidence *(26–31)*. Interestingly, HIV disease progression does not correlate tightly with specific MHC class II alleles that would be indicative of a role for CD4 T-cells in the immune response to this virus. One possibility is that chronic HIV infection with persistent viremia results in the anergy of all HIV antigen-specific CD4 T-cell clones, erasing the fingerprints of the subtleties of CD4 T-cell antigen recognition via the TCR/ peptide/class II MHC interactions during chronic infection.

The complex mechanism by which T-cells recognize antigen, in comparison with B-cell/antibody antigen recognition, has several important implications for responses to infectious agents and especially the development of vaccines. First, since antibodies recognize a broad range of conformationally dependent epitopes, whereas T-cells focus on only a limited set of peptide epitopes, the degree of crossreactive immunity to different quasi-species of the same infectious agent is often greater for T-cell immunity than for antibody responses. Second, owing to the special processing mechanisms required for T-cell recognition, especially the endogenous peptides loaded into class I MHC molecules for presentation to CD8 T-cells, live viruses may stimulate significantly different T-cell specificities than purified viral proteins administered in an adjuvant fashion. The use of live attenuated viruses as vaccines or the use of pseudotyped viruses or DNA vaccines have all been suggested as a practical means to circumvent this problem, in addition to other potential advantages. Finally, for antigens of limited structural heterogeneity there may be substantial variability between individuals based on MHC haplotype in responses to particular vaccines or infectious agents. To generate strong T-cell immunity, correlation of vaccine responses to particular MHC haplotypes may be just as important as inclusion of antigens derived from diverse clades of virus in vaccine development.

FUNCTIONAL HETEROGENEITY OF T-CELL SUBSETS

The T-cell repertoire can be defined by two distinct properties: the recognition specificity of the TCR heterodimer and the functional response of the cell after TCR stimulation. It is now clear that once a particular TCR heterodimer is expressed on the T-cell surface, the antigen specificity is frozen for all the clonal progeny of that cell. The functional responses available, however, are quite extensive and range from programmed cell death to initiation of distinct modalities of immune response. The complex mechanism by which the antigen specificity is determined (random rearrangement of two distinct polypeptides with both multiple germ-line gene segments and junctional diversity followed by selection for a relatively narrow band of avidity for self-MHC peptides) imposes special characteristics on the specificity repertoire.

The mechanisms by which the functional repertoire of T-cells is developed are less well understood, but they probably involve a similar strategy to that used during thymic

selection. The key element again is signaling generated from the TCR complex when it interacts with particular peptide/MHC epitopes. The TCR complex is not merely an on/off switch, but the avidity of interactions of the TCR with the myriad available peptide-loaded MHC molecules initiates biochemical cascades within the T-cell that are highly dynamic *(32).* If the avidity of these interactions is sufficient, cooperation among other adhesion molecules, including the major coreceptors CD4 or CD8, the adhesion molecule LFA-1 (CD11a), and the CD28 molecule, stabilizes this molecular binding in a process termed the immunologic synapse *(33).* This complex structure can deliver multiple levels of signal depending on the relative intensity and stability of the interaction. These multiple signals are most likely integrated at the level of multiple different promoter complexes, in which biochemical signals initiated at the cell surface are translated into the production of transcription complex components. In turn, these cellular signals can interact with multiple promoter motifs *(34),* resulting in coordinated patterns of expression of multiple unlinked genes (Fig. 2).

The proteins produced by such activated genes are of several classes, including those that initiate entry into the cell cycle; expression of unique cytokine receptors; expression of various effector cytokines; expression of new surface adhesion molecules; and new transcription factors. The products of this ensemble of gene activation interact in complex ways to determine not only the fate of that particular T-cell, but also the tempo of immune activation in the immediate microenvironment in which T-cell activation occurs. Expression of new cytokine receptors (e.g., interleukin-2Rβ [IL-2Rβ]) or inactivation of existing receptors (e.g., IL-12Rβ) can alter the subsequent response pattern of the responding T-cell. Many cytokines (i.e., IL-2, IFN-γ, TNF-α, IL-4, IL-10, IL-13, granulocyte/macrophage colony-stimulating factor [GM-CSF], and transforming growth factor-β [TGF-β]) serve as growth and differentiation factors for most other cells in the local microenvironment. These include other T-cells, dendritic cells and macrophages, endothelial cells, and B-cells, in addition to the responding cell via autocrine feedback. With a longer kinetic delay, activated T-cells change the pattern of cell surface adhesion molecules that alter the subsequent recirculation and tissue distribution properties of the cell. Investigators recognize some of these adhesion molecules as memory markers (such as CD44, CD62L, and CD45 isoforms), since their differential expression on previously activated T-cells allows detection by cell surface staining with available monoclonal antibodies.

Finally, and potentially most critical, initial T-cell activation can result in the production of new transcription factors, which may differentially affect the vigor of transcriptional activation on subsequent rounds of TCR-initiated signals. It is highly likely that such factors account for the significantly lowered antigen dose threshold required for full stimulation found in previously activated (memory) T-cells *(35,36),* compared with cells that have not been stimulated recently. Although many genes are activated in a coordinate manner, in individual cells some of the cytokine genes show distinct thresholds for activation based on different intensities of TCR/peptide/MHC stimulation. These distinct thresholds probably generate the significant clonal heterogeneity characteristic of antigen-specific T-cell activation *(37–41).*

This hypothetical scheme of T-cell activation (incorporating the functional subtleties of T-cell antigen recognition) contrasts with the more conventional views of classes of T-cells, based on static conceptions of antigen specificity, T-cell response, and

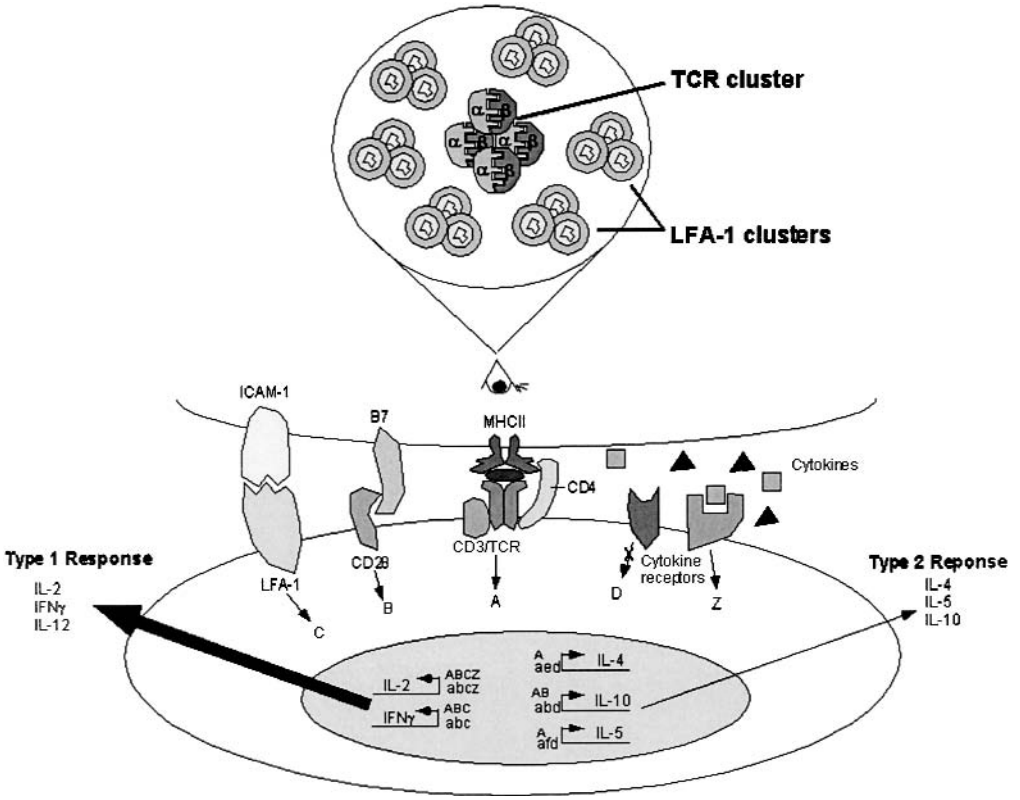

Fig. 2. Multiple distinct signal pathways converge to produce functional transcription complexes to allow coordinate activation of multiple genes. Alterations of the dominant pathway of signaling from successive cycles of antigen stimulation result in alternate pathways of functional differentiation. ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; IL, interleukin; LFA-1, leukocyte function-associated antigen; TCR, T-cell receptor.

memory versus naíve T-cells. First, antigen specificity is not a clean positive/negative phenomenon, even in response to a particular index peptide structure. All TCRs in the repertoire bind with modest avidity to ubiquitous self-antigen, and there is potential for anatagonist peptides that bind to the MHC restriction element well, but fail to stimulate the particular TCRs with high avidity for the index peptide. T-cells that interact with intermediate avidity to a particular index peptide/MHC complex may show some of the features of T-cell activation, particularly entry into the cell cycle if sufficient IL-2 is available, but they do not participate in the more stringent activation pathways.

Finally, the activation signals from engagement of the TCR reflect the product of the amount of peptide/MHC complex and the inherent TCR affinity. Thus, in the presence of high doses of a particular peptide, more T-cells (including those with slightly lower avidity) can become fully activated. In the presence of lower doses of the *same* peptide, (or in the presence of peptide antagonists), these same T-cells receive suboptimal signaling that not only results in failure to reach the threshold stimulus for full activation, but probably results in a different *kind* of activation. The phenotypic characteristics among the daughter cells of such qualitatively different kinds of antigen activation are probably

distinctive. Therefore, the sensitivity to TCR-initiated signal and the cytokine expression phenotype, as well as the pattern of adhesion molecule expression and tissue recirculation may all be different in these daughter cells. Thus, a static view of antigen specificity, which is implicitly defined by response to antigen, is not completely tenable, even though the actual structure of the TCR is not altered by antigen stimulation. Since the pattern of response is quite heterogeneous and dependent on the subtleties of formation and signal generation at the immunologic synapse, the range of peptides that a T-cell is specific for also depends on the circumstances of presentation and the life history of the particular T-cell. Finally, the simple dichotomy between memory and naíve T-cells is much too simple to classify different subsets of T-cells adequately. Not only is memory likely to be as heterogeneous as the response that is remembered, but many adhesion molecules used as markers of memory revert to a naíve status at different tempos.

The role of antigen dose in stimulation of responses may also be quite important in situations of persistent low-level antigen exposure, such as in chronic asymptomatic HIV infection. Persistent stimulation of high-avidity TCRs by a low concentration of HIV-derived peptides may result in exhaustion or anergy of relevant T-cells during chronic infection such that little CD4 helper activity is available for the CD8 T-cell response. A quick burst of a higher concentration of HIV peptides, such as might be experienced in a subject effectively treated with highly active antiretroviral therapy (HAART) who undergoes a scheduled treatment interruption, may allow the functional activation of these same cells to help mediate effective viral clearance. Thus, both the dynamics of antigen dose in vivo and the cytokine milieu in the histologic microenvironment may play critical roles in the ability to induce a functional immune response, beyond the mere presence of antigen-specific T-cells.

In addition to the heterogeneity that exists in concepts such as antigen specific/nonspecific and memory/naíve, considerable heterogeneity has long been recognized in the kinds of functional effector activity mediated by different classes of T-cells. There are multiple distinct cytokines that can be expressed after TCR activation in addition to the induction of two distinct pathways of direct lytic activity for target cells (the secretory pathway and the FasL/Fas interaction). There is significant heterogeneity in the pattern of individual cytokine gene expression, even within stable in vitro passaged T-cell clones *(37,38,41)*. Although there are patterns of cytokines that tend to be coexpressed, each individual promoter is under a unique pattern of control, with a distinct threshold for activation. Furthermore, there are multiple potential phenotypes, but any one T-cell usually has a very limited subset of these alternatives actually expressed. Not only are the subtleties of TCR/peptide/MHC interaction as discussed above critical for determining the assortment of particular functional activities with different TCR structures, but the cytokine milieu in which initial T-cell activation occurs plays a dominant role in segregation of the cytokine expression phenotype *(42–45)*. The role of the innate immune system in providing the bootstrap cytokines expressed in the local environment where particular antigen-specific cells become activated is probably critical in this process *(46–48)*. Thus, one can conceptualize the T-cell repertoire as a two-dimensional classification scheme, in which each particular specificity element sorts out into distinct functional categories dependent on antigenic stimulation experience.

The determination of how many distinct functional classes of T-cells exist in the repertoire is not clear. The history of cellular immunology has been characterized by

the continual subdivision of classes of cells initially thought to be homogenous (given a single name) into distinct categories based on newly discovered features. Lymphocytes have been separated into three distinct lineages and T-cells into sublineages based on both major coreceptor usage (CD4 vs CD8, which correlates with MHC restriction specificity) and distinct lineages that utilize distinct antigen receptors (αβ vs γδ TCR). To the first approximation, the CD4 and CD8 sublineages of T-cells are biased in the pattern of their functional differentiation to express particular patterns of cytokines (CD4 helper cells) or the induction of direct lytic activity (CD8 cytotoxic T-cells). However, exceptions to this dichotomy exist in both directions. There are CD4 T-cells that mediate direct lysis and CD8 T-cells that secrete cytokines mediating immunoregulatory activities.

There is also a further subdivision of T-cell subsets into functional classes based on the pattern of cytokine expression, the Th1/Th2 paradigm. Originally panels of murine CD4 T-cell clones were characterized that had distinctive cytokine expression phenotypes *(49).* Clones classified as Th1 express primarily IL-2, IFN-γ, and TNF-β (LT), whereas the Th2 cells express IL-4, IL-5, and IL-10. Further work has demonstrated that these sets of cytokines are associated with functionally distinct types of immune responses, establishing a link between T-cell phenotype development and cellular versus humoral immunity *(50–52).* In particular, IFN-γ is a potent macrophage-activating factor *(53,54)* and plays a critical role in delayed-type hypersensitivity (DTH) responses *(55,56),* whereas IL-4 and IL-5 are potent in B-cell growth and differentiation *(57,58).* Not only do these cytokines have distinctive biologic activities, but several lines of evidence also indicate that a reciprocal competitive relationship exists between cells with Th1 versus Th2 characteristics *(42,43,45,52,59).* Several infectious disease models have demonstrated the critical role of Th1 and Th2 cytokines in regulating the balance in favor of the host or the pathogen *(60–64).* Particularly clear-cut is the genetic susceptibility to *Leishmania* in mice. The disease course between inbred mouse strains is correlated with inherited tendencies to generate either a Th1 or Th2 response *(65–68).*

The original dichotomy of cytokine expression patterns has begun to blur into many individual distinct phenotypes based on differential quantities of expression within the classical phenotypes (variation in IL-2/IFN-γ ratio in Th1, and IL-4/IL-10/IL-5 and perhaps TGF-β in Th2 cells) *(69).* The multiplicity of functional phenotypes that have been characterized in different circumstances *(70–72)* suggests that the Th1 and Th2 designations do not represent true lineages (irreversible differentiation), but rather a useful initial distinction among a complex set of functional differentiation patterns. The general idea is that different patterns of cytokine gene transcription represent a primary functional distinction of different T-cell subsets.

Consideration of the significant heterogeneity of antigen-specific T-cell activation has several potentially important implications for the pattern of cellular immune responses to infectious agents and in particular to HIV-1 infection. First, the conventional method of measuring T-cell response to particular antigens used in most human disease clinical trials, the lymphocyte proliferation assay (LPA), is both more complicated than is usually thought and in some circumstances an unreliable guide to the potential for effective in vivo immune response. There are at least three kinds of cells required for a vigorous LPA response: 1) the availability of adequate APCs; 2) the existence of a few individual T-cells in each well that produce potent T-cell growth

factors (TCGFs); and 3) the low-threshold stimulation of other T-cells to grow in the presence of TCGF. In most circumstances, IL-2 produced by CD4 T-cells is the dominant TCGF, but other cytokines may play an important role in some situations. Although a strong LPA response correlates well with effective in vivo immunity to particular pathogens, weak or negative LPA responses can result from several different circumstances. These include deficient functional APCs in the population of blood mononuclear cells, clonal anergy (deficient IL-2 production) of relevant T-cell clones, production of alternative cytokines that inhibit T-cell growth, or a low frequency of functional IL-2-producing clones. Direct measurement of the frequency of individual T-cells that produce different effector cytokines shortly after antigen stimulation may yield more insight into the status of in vivo immunity than sole reliance on the conventional LPA response.

A second practical consequence of the complexity of T-cell immunity is understanding the mechanism of insufficient immune responses to certain pathogens, especially those that maintain persistent antigen loads during chronic infection. The conventional view is that such circumstances represent deletion of the small subset of antigen-specific cells, via clonal exhaustion or pathogen-specific infection (in HIV disease for the CD4 T-cell response). An alternative possibility is that persistent antigen load results in various alternative patterns of differentiation that fail to activate effective clearance mechanisms for the infection. T-cells with sufficient TCR affinity for peptides derived from the pathogen exist, but continual low-level stimulation anergizes these cells. In this context, the term anergy simply indicates that absence of the particular function is used as the index of response, not physical absence (clonal deletion) of the relevant cells. In some circumstances, immune deviation to produce Th2-like cytokines in contrast to the Th1 pattern somewhat accounts for such unresponsiveness. Examples include lepromatous leprosy *(60,73,74)* and the well-studied *Leishmania* major infection in mice *(65–75)*.

In the case of HIV-1 infection, although such classical immune deviation has been suggested *(76)*, an alternative possibility is that direct interaction of viral particles with the CD4 molecule together with persistent low concentrations of antigens yields T-cells with low-level TCR stimulation that fail to respond with high IL-2 production. The potential role of selection of viral variants that not only escape detection by particular T-cells but also produce peptide antagonists that block the responses to other epitopes and perhaps alter the cytokine expression pattern of reactive T-cells may also play an important role in some cases. As a consequence of functional anergy of T-cells with TCRs with high affinity for HIV-derived peptides, the infection may be controlled by helper-independent CD8 T-cells that are inherently inefficient. Since persistent low-level TCR stimulation may be required to sustain this pattern of differentiation, it may be possible to reverse this pattern by first eliminating most of the persistent viral antigen (by treatment with available potent antiretroviral drugs) followed by therapeutic immunization. If clonal deletion of HIV-specific T-cells during primary infection is responsible for the deficient CD4 T-cell responses in chronic HIV infection, the prospects for successful therapeutic immunization are fairly dim, given the low (but detectable) thymic output of new TCR specificities in adult humans *(77)*. If an as yet ill-defined anergic state exists among these critical cells, understanding the subtle mechanisms by which antigen can stimulate functionally distinct kinds of differentia-

tion may be critical to the design of effective therapeutic immunization.

ANATOMICAL DISTRIBUTION OF IMMUNE CELLS: RECRUITMENT TO INFLAMMATORY SITES AND REDISTRIBUTION

A final principle of the basic nature of T-cell-mediated immunity is the role of the anatomic distribution of immune cells in mediation of immune responses. First, unlike humoral responses in which the effector function of antibody is generally at a distant site from the antibody-producing cell, T-cell effector function is always localized to microenvironments directly associated with the active effector T-cell. The lytic function of cytotoxic T-lymphocytes (CTLs) takes place only in tight conjugates of the individual target cell and the CTLs, whereas cytokines are active only over short distances and act on other cells in the immediate tissue environment. In fact, a substantial portion of helper function for CTL formation is probably owing to the simple colocalization of activated CD4 and CD8 T-cells in the same tissue microenvironment caused by responses to the same antigenic entity, albeit to distinct peptides. This requirement for localized effector function results in the critical role of T-cell recirculation and recruitment to active inflammatory sites in the organization of in vivo T-cell-mediated immune responses. The development of a mononuclear infiltrate in a nonlymphoid tissue is the histopathologic hallmark of active T-cell immunity.

The ability to mobilize a sufficient number of T-cells to a local site is dependent on the constant recirculation of the low frequency of T-cells with a high-affinity TCR for a particular peptide/MHC epitope and the rapid recruitment of such cells. IFN-γ and TNF-α, as well as other cytokines produced by activated T-cells and macrophages, affect the local microvasculature, resulting in increased vascular permeability and the induction of vascular adhesion molecules. These adhesion molecules serve to facilitate recruitment of circulating T-cells into the microvascular bed surrounding the initial cytokine-producing cells. Although antigen-specific cells are preferentially accumulated in such inflammatory foci, most of the T-cells that accumulate in sites of inflammation do not have TCRs that bind with high avidity to available peptide/MHC complexes. Among the T-cells that are nonspecifically recruited to such sites are a few that reach a threshold of stimulation by the available peptide/MHC complexes and produce additional cytokines that amplify the nascent inflammatory focus. In addition, since T-cell activation occurs in such an inflammatory site bathed in cytokines such as IFN-γ and TNF-α, their pattern of antigen-activated differentiation is biased toward further production of these Th1-type cytokines. Control of the tempo of such iterative cycles of cellular recruitment and inflammatory cytokine production is probably the critical step in the overall intensity of T-cell-mediated immunity.

A corollary of these principles is that the population of T-cells in the blood may not be fully representative of T-cells that are actively involved in a tissue-localized immune response (Fig. 3). During periods of active T-cell immunity, such as localized responses to infectious agents in lymphoid tissue or responses such as solid organ transplant rejection, the blood is relatively depleted of antigen-reactive cells, owing to their sequestration in the local site of the active immune response. Although this is a relatively simple point, fundamental methodologic difficulties often produce subtle conceptual bias. To some extent, this conceptual focus on blood T-cells, simply because they are routinely available for analysis, is a contributor to the controversy concerning the interpretation

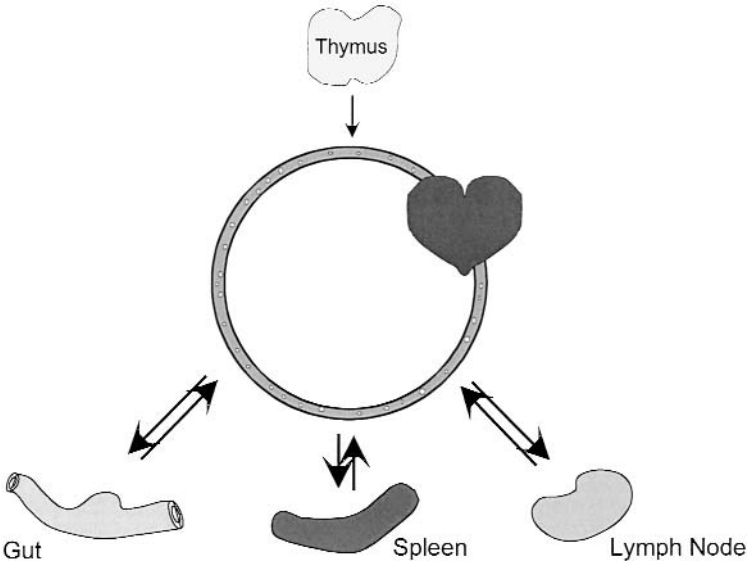

Fig. 3. The in vivo population of T-cells constantly recirculates to many different tissues. Local immune responses result in redistribution of T-cells to the site of immune activation and then nonhomogeneous distribution among body compartments.

of cellular changes after induction of HAART in HIV-1 infection. The initial proposal that the increase in blood CD4 T-cells after HAART was caused by an increase in total body T-cell number reflected the common use of the CD4 count (in blood) as a surrogate for total body T-cells. Although this relationship may be largely correct over the long-term natural history of HIV disease, short-term changes in blood lymphocyte numbers often reflect redistribution of cells between body compartments.

Some investigators proposed the alternative interpretation of a redistribution of cells early on *(78,79),* but the controversy lingers despite any direct evidence that the total body number of T-cells rises rapidly in any circumstance. Recent studies focused on comparison of lymphoid tissue and blood specimens before and after induction of HAART strongly indicate the reciprocal relationship of blood and tissue lymphocytes and the resolution of lymphoid tissue inflammation coincident with resolution of active viral infection of these tissues *(80).*

A similar line of reasoning cautions against overinterpretation of the relatively modest level of antigen-specific CTL effector function detected in blood T-cells during chronic HIV disease. Since the active infection exists primarily in the lymphoid tissue, the cells isolated from blood may have an inconsistent relationship with the level of active in vivo immunity during episodes of chronic infection. Together with the relatively difficult analytic procedure required to identify functional CTLs, tissue sequestration of active cells makes assessment of immunity using in vitro methodologies problematic. Ten years ago the failure to detect infectious virus in blood during prolonged asymptomatic chronic HIV infection led to the view of a dormant infection. The advent of sensitive viral RNA assays, together with evidence of rapid fall in viremia after induction of HAART, resulted in a conceptual shift: that there is rapid viral turnover throughout chronic infection. Similarly, the failure to detect robust immune

responses during chronic HIV infection using assays of blood T-cells does not indicate that viral replication is not controlled by active immune clearance mechanisms.

SUMMARY

This chapter has attempted to incorporate some insights from our current understanding of cellular immunity into an understanding of the pathogenesis of infectious diseases, with a primary focus on HIV disease. The complexity of T-cell recognition, with subtle functional consequences of particular MHC restriction elements, local milieu of activation, and kinetic profile of antigen dose, results in complex interactions between the immune system and persistent infectious agents. The interaction of ideas derived from basic biologic studies and development of workable therapeutic interventions is most productive when both basic and clinical investigators develop two-way communication. Incorporation of basic insights into new hypotheses that can be directly tested in infected humans offers an additional feature for clinical trial design beyond the availability of novel agents. Furthermore, development of an effective therapeutic strategy is often the key element in resolving fundamental questions of disease mechanisms, since effective interventions must be modifying key mechanisms in disease pathogenesis.

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