Immunologic Research 1999;19(2-3):107–118

Immunogenetics

The Role of Self-Recognition in Receptor Repertoire Development

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Abstract

The role of self-antigen recognition in the development of T and B cells of the adaptive immune system has been studied in several different ways. We have shown that CD4 T cells are selected on self-peptide:self-MHC class II ligands, and in the periphery, they are sustained by contact with the same or similar ligands. We have also observed that B cells are positively selected on unknown and presumed self-ligands. We have used this information to explore autoimmune diseases as well. Finally, we have recently identified the innate immune system as playing a crucial role in regulating expression of costimulatory molecules that are required for induction of adaptive immune responses.

Keywords

Self-recognition
T cell receptor
B cell receptor
Receptor repertoire
Idiotype
Autoimmune disease
Innate immunity

*People whose work is included in this review are as follows: Chuck Annicelli, Joanne Appicelli, Avlin Barlow, Jody Baron, Michael Carrithers, Chaoqun Chen, Alexander Chervonsky, Beth Cohen, Bonnie Dittel, Tori Evans, Jennifer Granata, Fridrika Hardardottir, Xin He, Jon Kaye, Matthew Levine, Yang Liu, Grigori Loysev, Ruslan Medzhitov, Paula Preston-Hurlburt, Eve Robinson, Alexander Rudensky, Derek Sant'Angelo, John Shanabrough, Christophe Viret, Irene Visintin, and Sue Wong

Introduction

The overall focus of work in my laboratory in the Section of Immunobiology at the Yale University School of Medicine is how the adaptive immune system discriminates self from nonself. Thus, we study how receptors on cells of the innate immune system play a key role in distinguishing infectious nonself from noninfectious self, the development, selection, and onward survival of cells bear-

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Humana Press Inc.
0257–277X/99/
19:107–118/$13.00

ing a mature repertoire of receptors in adaptive immunity, and the role that T and B lymphocyte receptors play in the autoimmune diseases insulin-dependent diabetes mellitus (IDDM) and experimental allergic encephalomyelitis (EAE).

The Role of the Innate Immune Response to Pathogens in Controlling the Adaptive Immune Response

The activation of mature naive peripheral T cells requires costimulation by molecules presented on the same cell that delivers the antigenic signal to the T cell receptor *(1)*. Once the mature T cell receptor (TCR) repertoire is established on self-peptide–self-MHC complexes (*see* Next section), how do the T lymphocytes know which foreign antigens to respond to? This question is central to all studies of adaptive immunity, but the answer to it appears to be housed in the innate immune system. This system is very ancient, since fragments of it are found in plants, invertebrates and vertebrates. It is believed, in vertebrates, to provide receptors that induce the costimulatory molecules used by T cells to decide whether a peptide is derived from an infectious agent and should lead to an adaptive immune response, or from noninfectious self-molecules that should not activate adaptive immunity. We study this process using a combination of mammalian and insect systems, which has helped us to analyze both systems. These studies have largely been carried out by Ruslan Medzhitov, with help from two extremely talented technicians, Chaoqun Chen and Paula Preston-Hurlburt, and from the laboratories of Sankar Ghosh, who studies the role of nuclear factor-kappa B (NF-κB) in innate immune responses (*see* Ghosh, this vol., p. 183), and of Jules Hoffman in Strassburg, France, who studies host defence in the fruit fly *Drosophila melanogaster*.

In flies, which have no adaptive immunity, the innate immune system is the only mechanism available for host defense. Therefore, it is much easier to study innate immunity in flies than in vertebrates. Recently, Hoffman and colleagues showed that flies deficient in several genes involved in dorso-ventral pattern formation were also unable to resist infection with fungal spores. The most upstream member of this system that could be implicated in the defective response to fungal spores was the gene encoding the pro-Spätzle molecule, but genes upstream of this point that encode proteases involved in dorso-ventral pattern formation were not involved, implying a branch point upstream of Spätzle between the antifungal response and the dorso-ventral patterning which occurs in embryonic flies. However, both pathways proceed via the Toll protein, and use identical elements downstream of Toll to signal via dorsal or closely related proteins homologous to NF-κB *(2)*.

We decided to investigate whether a similar system exists in mammals. We first explored the expressed sequence tag (EST) database for Toll-like molecules. We cloned a gene with a high degree of homology across its entire length to the drosophila Toll protein, which we called hToll *(3)*. The predicted structure of the hToll protein was that its ectodomain would be made up of leucine-rich repeats, with a membrane-proximal sequence that contained four half cystine residues in the exact same location as the fruit fly gene dToll. It was known that disrupting any one of these four cystine residues produced a dominantly active form of the dToll gene, so we constructed a dominantly active hToll gene by removing three of the four half cystines and replaced the ectodomain with the ectodomain of mouse CD4 to allow ready detection of transfectants. This indeed conferred dominant activity on the hToll gene, allowing us to ask what genes were activated in cells transfected with the CD4-hToll construct.

We showed that the dominantly active hToll gene induced the activation of NF-κB, and

using reverse transcriptase polymerase chain reaction (RT-PCR) that activation of NF-κB induced the expression of pro-inflammatory cytokines, such as interleukin 1β (IL-1β), IL-6 IL-8, and p40 of IL-12, and of B7.1 and B7.2, the main costimulatory molecules for T cells. We have subsequently studied both the mammalian and the fly Toll signaling cascades. In mammals, we discovered that a known molecule called MyD88, which was previously cloned as being involved in myeloid differentiation, could also serve as an adapter protein that interacted with the cytoplasmic domain of Toll and the IL-1 receptor (IL–1R) called a Toll-IL-1R (TIR) domain. MyD88 itself is made up of two domains, a TIR domain and a so-called death domain (DD), both of which are involved in protein–protein interactions. MyD88 is downstream of Toll and upstream of the serine/threonine innate immunity kinase (SIIK) known as IL-1R-associated kinase (IRAK) *(4)*. During our studies of IRAK, we have identified two other SIIK molecules, one containing a caspase-associated recognition domain (CARD) domain, which we have named CCK for card-carrying kinase (Medzhitov and Janeway, unpublished results). This SIIK is downstream of an unknown receptor, perhaps one of several Toll homologs in the human, since there are at least 12 Toll-like genes defined in humans already.

In mammalian cells, the next molecule in the signaling cascade is TRAF-6, which has an unknown function in this pathway, but is presumed to mediate the interaction between IRAK and NF-κB-inducing kinase (NIK). NIK in turn activates two related proteins that form a heteroimeric structure called IκKα and β, which, on phosphorylation by NIK, are activated to phosphorylate the inhibitory protein IκB that is bound to the transcription factor NF-κB in the cytosol, holding it prisoner. On phosphorylation of IκB, it rapidly dissociates from NF-κB and is degraded in proteasomes, releasing NF-κB to enter the nucleus and activate genes involved in adaptive immunity. This system is thus key to understanding how the adaptive immune response to foreign, pathogen-associated molecules is activated.

It turns out that the same or similar elements exist in insect cells, since we have found a drosophila homolog of TRAF-6 playing a critical role in interacting with a protein that we call Cactus kinase. Cactus kinase is most homologous to IκKβ, but so far is the only known kinase that can phosphorylate the IκB homolog, which is called Cactus in the fruit fly. It may well exist as a homodimer that has been duplicated and diversified in mammals.

Having found all of these analogies between vertebrates and invertebrates, we then asked if similar proteins are found in plants, and if so, are they involved in host defense of plants? Indeed, scanning the literature for homologies, it turns out that a series of genes in plants are also involved in host defense and all of them are homologous to the fruit fly and vertebrate defense systems. However, since these were all isolated by positional cloning of disease-resistance genes, there was no bias for isolating Toll-like molecules.

Thus, it appears to us that the presence of the Toll signaling cascade is a universal mechanism for host defense in vertebrates, invertebrates, and plants, making it a very ancient system of host defense against infection. How it is used in each situation is different depending on the sources of the threat and the mechanism of response, but the basic building blocks are all in place in all multicellular organisms, and are used for the same purpose to mediate the response to infectious agents (Fig. 1). In mammalian systems, this innate immune response also triggers the synthesis of costimulatory molecules, thus providing a link between the innate immune response to infection and the adaptive immune response to the same infection, which is almost totally dependent on the expression of these costimulators *(5)*.

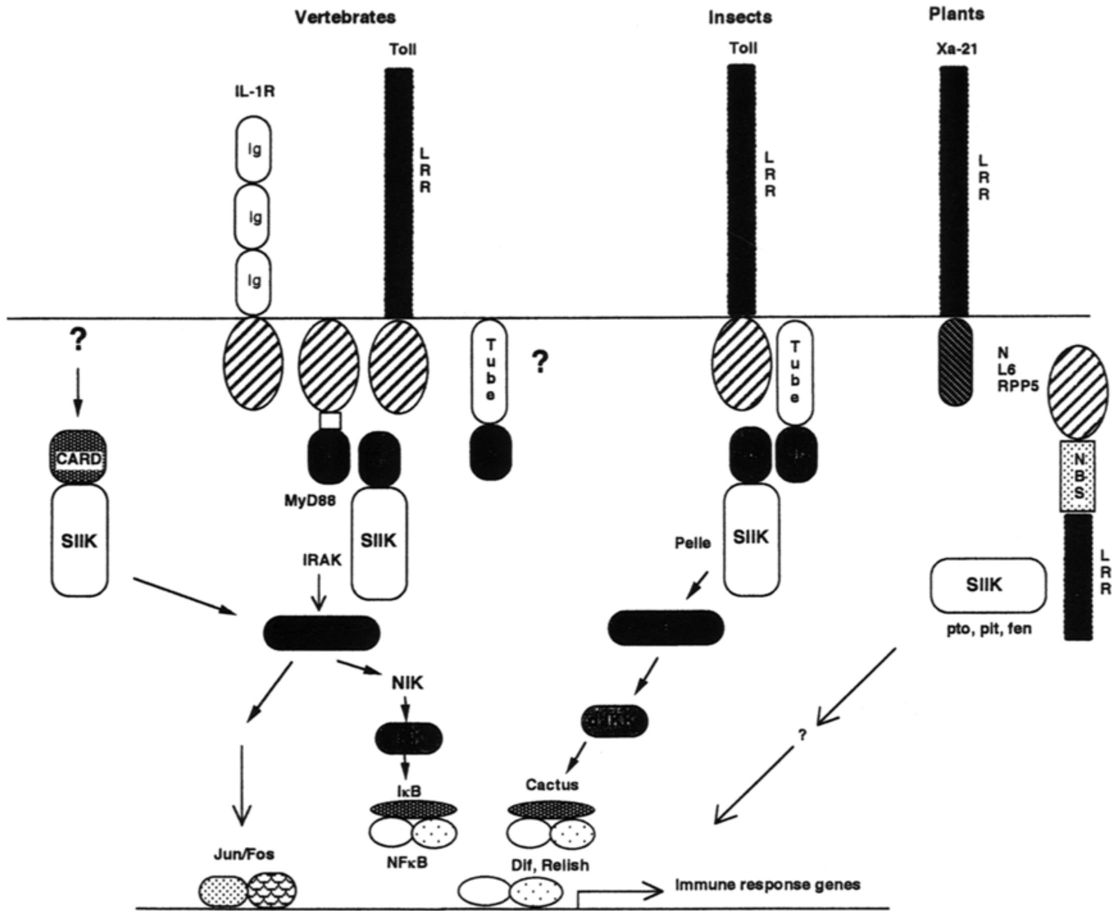

Fig. 1. Ancient system of host defense This figure illustrates the similarity of host defense systems from plants to invertebrates to vertebrates. All have certain common features, particularly in their fundamental protein domains. These are illustrated in various shapes, and appropriate initials. Leucine-rich repeat (LRR); Toll-IL-1 receptor (TIR) striped ovals; death domain (DD); serine/threonine innate immunity kinase (SIIK); TNF receptor associated factor (TRAF); IκB kinase (IKK); IκB, the inhibitor of NF-κB.

Development and Selection of Mature Receptor Repertoires

Our work on T and B cell development and the rearrangement of receptor genes began by chance when Derek Sant'Angelo observed that mice with a transgenic TCR α chain had variability in the TCR β chain junctions of antigen-specific T cell hybrids, whereas mice with a transgenic TCR β chain had constant TCR α chain amino acid sequences on immunization with wild-type and various mutant peptides *(6)*. This could be interpreted in several ways, but the evidence supported an event that preceded immunization, so we examined the preimmune repertoire in several TCR β chain transgenic mice. We found that the mature TCR repertoire in TCR β chain transgenic mice was largely determined by the prerearranged transgenic TCR β chain *(7)*. We were also able to demonstrate in two different β chain transgenic mice that even nucleotide sequences that did not yield the identical amino acid sequence as the clone

that gave rise to the TCR β chain (the parental clone, leading to the designation of this TCR α chain as parental) could still direct recognition of the immunizing peptide. Indeed, we observed that 13/15 amino acid sequences would yield responses to a peptide of conalbumin that were of nearly identical intensity to that made by the parental TCR, and 6/15 were identical to the parental sequence at the amino acid level (*7*; Robinson, unpublished data). We also showed that these sequences were frequent in both peripheral $CD4^+$ nonimmunized T cells, and $CD4^+$, TCR^{hi} cells in the thymus of the same mice. However, the origin of these cells remained obscure.

To determine if peptides were involved in this putative positive selection event, we turned to mice reported by others *(8–11)* to have random TCR repertoires, but a strong propensity to form receptors that recognized directly the MHC class II allele on which they were raised. These mice were prepared by deleting the gene for H-2Mα, whose protein product (H-2M) is necessary to remove the peptide known as class II invariant chain peptide (CLIP) from the MHC class II molecule, $I\text{-}A^b$. Thus, the dominant selecting peptide was CLIP in these mice. The observation that these mice had diverse TCR repertoires was based on the finding that they had a random assortment of TCR β chains as determined by anti-Vβ antibody staining. However, when we constrained their choice of β chains to one, encoded by the same TCR as we had used in the prior analysis, we made a series of observations that convinced us that the selected TCR repertoire was highly constrained by being selected on the CLIP peptide bound to $I\text{-}A^b$ *(7)*. The first of these observations was that the length of the TCR α chains was random in TCR β chain transgenic mice with a random assortment of self-peptides, but over 80% of the sequences were of the same length in mice having a transgenic TCR β chain and deficient in H-2M. Furthermore, in non-TCR transgenic mice deficient in H-2M, we also observed a random set of TCR α chain sequences in Vβ8.2-positive T cells. The second was that the number of T cells in the thymus and the lymph nodes was identical in H-2M-deficient mice with random TCR β chains and H-2M mice that bore a transgenic TCR β chain, owing no doubt to 100% success at the process known as β selection in TCR β chain transgenic mice. The third was that the frequency of cells reactive to $I\text{-}A^b$ bearing a normal set of self-peptides was identical in TCR β chain transgenic, H-2M-deficient mice and normal H-2M-deficient mice, indicating that the previous finding of excess reactivity to $I\text{-}A^b$ was independent of TCR β chain diversity. The fourth observation was that the TCR α chain sequences of cells prior to positive selection was random, whereas TCR α chain sequences after positive selection were highly constrained. All of these findings led to the conclusion that the nature of the self-peptides presented to developing thymocytes determined the structure of the mature TCR repertoire, both through positive selection as established by us for CD4 T cells and by others for CD8 T cells, and through negative selection as established by many other workers in the early years of TCR transgenic mice. We have also observed (Viret, unpublished), as have others *(12–14)*, that T cells bearing various TCR as transgenes that are well selected on $I\text{-}A^b$ are not selected on $I\text{-}A^b$ in an H-2M-deficient thymus. This provides further evidence for the role of peptides derived from self on positive intrathymic selection.

We have gone on to analyze in detail positive intrathymic selection by sorting thymocytes from a different TCR β chain transgenic mouse. This mouse, which was produced by Joan Goverman at the University of Washington, bore a TCR β chain that was derived from a T cell hybrid specific for the peptide Ac1-16 bound to the MHC class II molecule $I\text{-}A^u$. The

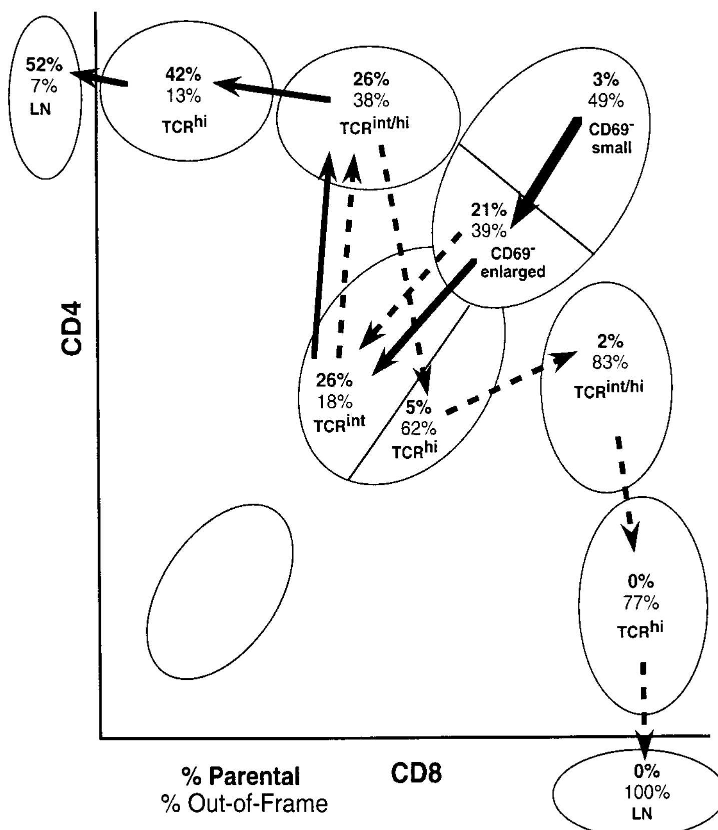

Fig. 2. A molecular map of T cell development The route traveled by a thymocyte during its maturation to a peripheral T cell is shown, beginning with the population of double negative cells, and progressing via various subsets, as shown. The figures in bold type are identical to the parental clone at the amino acid level, while the figures in plain type are out-of-frame. The balance of 100% is in-frame sequences that are different from the parental sequence at the amino acid level. Bold arrows give the maturational pathway of cells destined to become CD4, TCR^{hi} cells and the dotted arrows show the route followed by cells destined to become CD8, TCR^{hi} cells.

α chain in the parental hybridoma is formed at high frequency by direct joining of $V\alpha$ to $J\alpha$, allowing this junction to serve as a molecular marker of positive selection *(15)*. The results of this analysis have given us the scheme illustrated in Fig. 2. The data summarized in Fig. 2 give the percentage of parental joins in bold face type in each subpopulation of T cells in the thymus; the figures in plain type are the percentage of out-of-frame α chain joins. It is apparent that the first population to show positive selection is the $CD4^+$, $CD8^+$, $CD69^-$, TCR^{lo} cells that have undergone enlargement. These cells progress to the $CD4^{lo}$, $CD8^{lo}$, $CD69^+$, TCR^{int} population very rapidly, which then resynthesize their CD4 coreceptors about 1 d before starting the resynthesis of their CD8 coreceptors, as shown previously by our collaborators on this project, Bruno Lucas and Ron Germain. As a result, the populations are made up of a mixture of precursors of CD4 T cells and CD8 T cells, until these precursors divide into distinct lineages after the $CD4^+$, $CD8^{lo}$, $CD69^+$, $TCR^{int/hi}$ stage of intrathymic development, as shown in Fig. 2. This work was greatly assisted by the efforts of a Yale

undergraduate working in the lab, Beth Cohen, who made the initial observation that TCR^{lo} thymocytes had random α chain sequences, but TCR^{hi} thymocytes had almost uniform TCR α chains that were parental in sequence. The high frequency of parental α chains also allowed us to demonstrate that unimmunized T cells from these TCR β chain transgenic mice could respond strongly to the Ac1-16 peptide of myelin basic protein (MBP) to which the parental T cell hybrid also responded. Thus, in this case, the self-peptide that is presumably selecting this TCR is very powerful; this was originally suspected from the intense positive selection seen in αβ transgenic mice of this and a second TCR, prepared by my student Jody Baron in collaboration with Susumu Tonegawa and Juan Lafaille, and further analyzed by Fridrika Hardardottir during her studies. These two transgenic receptors show intense positive selection in $I\text{-}A^u$ mice, such that no true $CD4^+$, $CD8^+$ double-positive cells are produced, and all thymocytes show either a $CD4^+$ single positive phenotype or a $CD4^{lo}$, $CD8^+$ phenotype. These latter cells are destined to die within the thymus, but they can be rescued to mature into $CD8^+$ single positive thymocytes by using a bcl-2 transgene, although such cells never exit the thymus (Hardardottir and Cohen, unpublished observations).

We are continuing to explore positive intrathymic selection in several different TCR β chain transgenic mice, as well as onward survival in a model being analyzed by Christophe Viret, in which an αβ TCR transgene is used to follow the fate of T cells in the mature pool that emerges in the periphery. These results show that the T cell maintains its conversation with self-peptides and self-MHC molecules that interact with its receptor after it leaves the thymus, and this presumably involves ongoing signals for survival after thymic exit (Viret and Janeway, in press).

Although everyone agrees that T cells undergo positive intrathymic selection on self-MHC molecules, as proven in a number of different ways, the question of whether B cells show similar behavior is not answered. It was known that B cells do not recognize nonself-MHC molecules with the elevated precursor frequency that characterizes T cells, so the ligand that could select B cells is a mystery.

In an attempt to ask whether a positive selection event occurs in B cells, Matt Levine performed the following experiments in collaboration with Mark Shlomchik (*see* Wang and Shlomchik, this vol, p. 261). Using immunoglobulin heavy chains as transgenes, by analogy to the studies described for T cells using transgenic TCR β chains, Levine analyzed the usage of κ chains in B cells of the immature (HSA^{hi}, IgM^{++}, IgD^-) short-lived B cells and the mature, long-lived B cell (HSA^{int}, IgM^+, IgD^+) populations. He observed that there was strong evidence for positive selection of B cells bearing particular Vκ sequences during this transition. This implies that the transition from the short-lived immature B cell to the longer-lived mature B cell population, during which 70-90% of the B cells die, is the B cell equivalent to positive intrathymic selection during T cell development. This transition is normally accompanied by a subtle decrease in the level of surface IgM, which may indicate that these B cells are having their receptors ligated to generate subthreshold levels of signals. This could be the survival signal defined by Goodnow *(16)* that occurs during passage through the lymphoid follicles. A similar signal was shown by Lam, Kuhn, and Rajewsky *(17)* to be delivered via the receptor. We are examining two hypotheses to explain these results. The first is that this signal is delivered by contact with environmental antigens, perhaps in the gut, and the second is that the signals derive from self-antigens, perhaps via the idiotypic network of

Jerne *(18)*. Whether either of these has validity is the subject of ongoing investigation.

The Role of T Cell Receptors and MHC Molecules in Autoimmunity

My lab also studies two autoimmune diseases, mainly by making cloned T cell lines that will recapitulate the events in spontaneous or induced disease on adoptive transfer to normal, syngeneic hosts. In each case, we are trying to figure out the immunopathogenesis of the disease as a prelude to devising strategies for curative therapy or preferably prevention by vaccination against disease. The diseases we study are mouse models of important human autoimmune diseases: multiple sclerosis is studied using an established animal model called EAE, whereas IDDM is studied in the nonobese diabetic (NOD) mouse. These mice develop diabetes spontaneously starting at about 14 wk of age.

In both of these autoimmune diseases, we have initially created cloned of T cell lines that adoptively transfer the disease efficiently and at low doses of cells. The CD4 cloned T cell line that transfers EAE is called clone 19; in this case, we have produced a mouse that carries the TCR of clone 19 as detected by staining with a monoclonal antibody (MAb) called 19G that is directed at its TCR *(19)*. The cloned T cell line that adoptively transfers diabetes is a CD8-bearing T cell named G9 *(20)*. G9 uses a TCR that is encoded in Vβ6, and can be detected in tissue sections of islets in irradiated host mice by staining for Vβ6 and CD8. We presently have Vβ6 TCR transgenic mice which we are using to analyze the TCR repertoire for the α chain of the T cell clone, but we do not have either a clonotypic antibody or the α chain as a transgene. Although we know that the antigen in the case of clone 19 is the myelin basic protein peptide Ac1-16, used earlier in the analysis of T cell development and selection in the thymus, we are still trying to determine the antigen recognized by clone G9. This is predicted to be a nine amino acid peptide, since it is presented by the major histocompatibility complex (MHC) class I molecule K^d. We are presently attempting to identify this peptide by a novel strategy using T cell hybridomas that produce β-galactosidase, which are detected by turning blue when they bind to their cognate ligand.

Pathogenesis and Prevention of EAE

In the case of EAE, we have been studying the pathogenesis of this disease for several years. First, we defined the molecules on the cell-surface that were necessary to produce disease. The molecule known as α_4 integrin was crucial for invasion of the brain in irradiated recipients. This was explained by the appearance of its counter ligand vascular cell adhesion molecule-1 (VCAM-1) on the blood vessels in the brain *(19)*, but how did the initial cell enter the central nervous system to induce these adhesion molecules, which are absent from the endothelial cells of the brain, and was α_4 integrin essential for this initial cell to enter the brain? The answer appears to be that activated lymphocytes of any specificity enter the brain all the time, but only those with specificity for a brain antigen persist in this site. For want of a better term to describe this behavior, we have adopted the term "blundering" to account for the ability of cells to get into the brain (and presumably other tissue sites as well). The activated T cells that blunder into the brain would have their normal counterparts during the response to infection, with infection in the brain being a real threat to survival, especially those infections that do not stimulate local inflammation. When nonantigen-specific activated T cells are infused into mice, there is a brief entry of activated T cells across the blood–brain barrier which nearly disappears after 24 h. However, when cells specific for MBP Ac1-16 but lacking the α_4 integrin needed to produce

disease are transferred to irradiated syngeneic mice, they enter the brain as efficiently as the nonantigen-specific cells, but unlike these former cells, they remain in the brain tissue for at least 24 h and induce the expression of VCAM-1. Thus, our current hypothesis for the pathogenesis of EAE leading to infiltration of autoimmune T cells into the brain is that the initial antigen-specific cells that may or may not have α_4 integrin cross the blood–brain barrier and initiate the inflammatory process. This leads to sustained disease only in the presence of autoreactive T cells expressing α_4 integrin on their surface. We have also shown, in collaboration with the group of Joe Madri of the Department of Pathology, that α_4 integrin activates matrix metalloproteinase 2 (MMP2), which allows the recruited cells to penetrate the vascular basement membrane with high efficiency. The original "blundering" step uses binding to P-selectin as one part of the mechanism of crossing the blood–brain barrier as shown by my postdoctoral fellow, Mike Carrithers.

In mice bearing the transgenic TCR from clone 19, no spontaneous disease is observed, at least in our mouse room, which is maintained as a closed colony. However, when the TCR is bred to mice that lack all lymphocytes owing to deletion of one or other of the RAG proteins, or DNA-PK_{cs}, disease occurs spontaneously *(21)*. The lack of disease in mice that carry the transgene and are able to rearrange endogenous receptors is owing to CD4 T cells *(22,23)*. Thus, to understand the spontaneous activation of these T cells, we are attempting to clone a new class of cells called regulatory T cells, or Tr1.

In an attempt to prevent spontaneous disease, we are collaborating with the group of Howard Weiner at the Brigham and Women's Hospital at Harvard Medical School in studies of oral tolerance. We have already demonstrated that mice carrying the clone 19 TCR are highly susceptible to active disease when challenged with MBP Ac1-16 peptide, and their susceptibility is markedly reduced by feeding myelin basic protein containing this peptide. It was also demonstrated that this was owing to the secretion of the antiinflammatory cytokine transforming growth factor β in response to culturing with the Ac1-16 peptide of MBP and such cells bear the 19 G clonotypic receptors *(24)*. We are also using this model to screen various drugs proposed to alleviate brain inflammation.

Pathogenesis and Prevention of Autoimmune Diabetes in NOD Mice

In our studies of diabetes in the NOD mouse model, we have made a number of findings that we believe will be important. First, we have found that diabetes can be accelerated by providing the islets with a transgene-expressed costimulatory molecule, human B7.1. This allows us to test both the role of particular cells in accelerating diabetes, and to rule in or out various hypotheses of diabetes pathogenesis. In a recent paper, we have shown that B cells appear to be important as antigen-presenting cells, since absence of B cells completely inhibits spontaneous IDDM, but in the presence of islet-expressed costimulatory molecules, diabetes in the absence of B cells occurs at the same rate as in normal RIP-B7.1 transgenic mice. On the other hand, in the absence of MHC class I molecules, produced by deletion of the molecule β_2 microglobulin, diabetes does not occur, even in the presence of islet-expressed B7.1. Finally, the role of CD4 T cells appears to be more complex and more difficult to analyze. In mice that lack expression of the CD4 molecule, IDDM does not occur spontaneously, but it does occur at a retarded rate in the presence of islet-expressed B7.1. Thus, our studies have provided strong evidence for a critical role for CD8 T cells, a less crucial role for CD4 T cells, and a role for B cells as antigen-presenting cells, since demonstrated by the ability of the

human B7.1 transgene to overcome the inhibition of IDDM seen in NOD mice lacking B cells *(25)*.

We then set out to see if we could make β cells resistant to rejection by allogeneic T cells as a prelude to using the same approach to render β cells resistant to xenogeneic rejection. Our thinking was driven by the previous findings by Bellgrau et al. *(26)* and Griffith *(27)* that immune privilege was a function of surrounding cells with Fas ligand (FasL). Alexander Chervonsky, a former postdoctoral fellow in the lab and a current member of the Jackson Laboratory in Bar Harbor, ME, had the idea that FasL driven by the β cell-specific rat insulin promoter-1 (RIP-1, also used to express the B7.1 molecule in β cells) could render such mice resistant to spontaneous IDDM. Unfortunately, this did not occur. However, careful study of these mice revealed that this transgene did not protect β cells from destruction because the β cells could express the TNF receptor family protein known as Fas. When Fas appeared on the β cells, it was immediately engaged by FasL expressed by the β cells with the consequence that the β cells committed suicide. The induction of Fas could be accelerated by exposure to clone G9 cells, which express both FasL and interferon γ (IFN-γ), which is thought to induce expression of Fas on β cells. When G9 cells were administered to mice that did not express Fas owing to the mutation known as *lpr,* they were completely resistant to the lytic effects of G9 *(28)*. Thus, we are now planning to design a different transgene to be driven by RIP-1, in which a mutant form of the Fas protein is placed upstream of an internal ribosomal entry site (IRES) followed by the same FasL gene used in the original RIP-1-FasL construct. This work is proceeding in conjunction with Chervonsky and Flavell (*see* Flavell, this vol., p. 159). If such mice are able to donate their β cells to any strain of mouse or rat, we will then make the same construct from the human equivalents of Fas and FasL and insert it into a large domesticated animal, such as a goat, in order to test its utility in human xenografting, since β cells are usually transplanted as cells or bits of islets, the problem of antibody-mediated attack on endothelial cells may be reduced (see Bothwell, p. 235 and Pober, p. 225, this vol.).

A better way of treating diabetes in humans would be to prevent it altogether. This is the long-term goal of our work in this field, and we are focusing on immunization with insulin, since feeding insulin is said to prevent diabetes in NOD mice by an immunological response consisting of secretion of transforming growth factor β (TGF-β) *(29)*. This is reinforced by a recent paper from the laboratory of Sherwin who has an insulin-specific CD4 T cell clone that secretes TGF-β and protects the β cells and islets from autoimmune attack *(30)*. We are trying to design a safe and simple vaccination technique that will reliably produce insulin-specific cells that secrete TGF-β by epicutaneous administration of insulin in collaboration with the laboratory of Kim Bottomly (*see* Metz and Bottomly, p. 127, this vol.). If this can be achieved in mice, we plan to extend it to people in hopes of producing a sufficiently safe and effective vaccine that can be used universally. The author realizes that he will not live to see this day, but is hopeful that someone will one day vaccinate all the world's children not only to prevent infectious diseases, but also against immunological diseases like IDDM.

The Central Role of Self-Peptide–Self-MHC Complexes in Adaptive Immunity

All adaptive immune responses that depend on T cell activation depend in turn on the recognition of a complex of a peptide–MHC complex. When the peptide is derived from a foreign antigen, the response is directed against the foreign antigen, and it is usually but not always associated with protective immunity.

However, when the response is directed against a self-antigen, then it usually is one that is effective at positively selecting the particular T cell and sustaining it in the peripheral lymphoid tissues. Much the same occurs with B cells, but in the case of B cells, we do not yet know the nature of the self-antigen that ligates the receptor on mature B cells. This theme links our studies of autoimmune disease to our basic studies of T and B cell development, and gives meaning to our studies of the regulation of costimulatory signals via the innate immune system. Basically, the author believes that the cause of the common finding of immunodominance and of MHC-linked susceptibility to particular autoimmune diseases *(31)* is the positive selection of T cells on self-peptide–self-MHC molecular complexes. These drive the positive selection of T cells and particularly of T cells capable of causing autoimmune disease, and they play a further role in sustaining the cells once they leave the thymus and enter the periphery. This lesson is very counterintuitive, but I am convinced that it is correct. In the future, together with the study of innate immunity, it will become the central question on which the author's laboratory will focus in the future.

Conclusion

I have tried to present the full range of issues that we study in my laboratory, but I realize that I have only scratched the surface. There are two reasons for this. The first is that the people who work with me are all highly talented and are usually better informed on their particular project than I am. The second is that if I tried to describe all the projects performed by any one postdoctoral fellow or graduate student, I would be able to write a full article on each person's research. Therefore, with apologies to all the people listed on the title page, this description will have to do.

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