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－Review Article－

Immunological Tolerance, Autoimmunity and Host Defense

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Rearrangement of the antigen-specific lymphocyte receptor genes generates repertoire of T and B cells that can attack any emerging pathogens as well as host tissue itself. This *horror autotoxicus* has to be removed by mechanisms collectively called self-tolerance. Recent advances in immunological science revealed that there are multiple mechanisms of tolerance both in central and peripheral organs. However, it has become more and more apparent that cellular mechanisms that induce tolerance (inactivation) and activation of lymphocytes are quite similar. Possible mechanisms by which immune system distinguishes self-structure from pathogens will be discussed.

Key Words: T cell receptor (TCR), major histocompatibility antigen complex (MHC), antigen presenting cells (APC), tolerance, transgenic mice,

Introduction

Immune system is designed to combat against the invasion of varieties of microbes into the body. This system has been evolved over many years adapting to the alterations in the environmental microorganisms and preparing for the emergence of any novel pathogens. The main cell types of the immune system, which highly evolved for this purpose, are T- and B-lymphocytes. During the initial phase of the lymphocyte development, rearrangement of the antigen receptors in lymphocyte precursors occurs randomly, creating a huge repertoire of lymphocytes each expressing a distinct antigen-receptor. As a result, these lymphocyte precursors develop into cells that can recognize self-structure as well as pathogens. Therefore, the expense to prepare dealing with any unexpected pathogen is the possibility of attacking its own tissue. It is necessary to deplete or inactivate lymphocytes that recognize self-tissue before it attacks their own tissue to prevent self-destruction. This process, which is called immunological self-tolerance, occurs during the generation of the lymphocytes in the primary lymphoid organs (central tolerance) as well as in peripheral tissue (peripheral tolerance). This review deals mainly with the current understanding of the mechanisms of T cell tolerance in relation to the host defense against invading pathogens. I will mainly focus on the issues on the peripheral tolerance, which is not completely revealed but is important for our understanding of how immune system distinguishes pathogen-derived antigens from bunch of self-antigens.

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T cell antigen recognition and activation/inactivation

Unlike B cells, which recognize conformational structure of the antigen by immunoglobulin receptor on cell surface, T cells are designed to recognize peptides buried in the groove of MHC expressed on antigen presenting cells (APC). This difference in antigen recognition between B and T cells derives from the difference in the location of the target microbes that they recognize; T lymphocytes attack pathogens associated with cells, while B-lymphocytes are designed to produce antibodies that attack microorganisms outside of cells. The requirement of the recognition of cell-associated pathogens resulted in the presentation of antigen in association with the major histocompatibility antigen complex (MHC) to T cells. In this way, T cells can recognize pathogens invading inside of cells. There are two types of MHC, class I and class II; MHC class I molecules associate with peptides derived from cytoplasm while MHC class II associate with peptides derived from molecules engulfed by phagocytosis or endocytosis. T cell receptor (TCR) is designed to recognize peptides associated with MHC. Thus, T cells does not have to be tolerized to all self-structure. It only needs tolerized to peptides

buried in the MHC groove.

Professional Antigen Presenting Cells

When T cells recognize the right peptide/MHC on APC, they are activated and differentiate into functional T cell subset, or deliver their effector function such as cytokine secretion and cytolysis. However, naive T cells, which are mature but have not been encountered the relevant peptide/MHC in the periphery, cannot be activated by the recognition of peptide/MHC alone. They need a second signal delivered by APC in addition to a TCR-mediated signal for full activation (Fig. 1). It is believed that T cells become unresponsive to subsequent stimulation if they recognize right MHC/peptide without delivery of the second signal[1]. Those APC, which can activate naive T cells, are called professional APC. They include dendritic cells, activated macrophages and activated B cells.

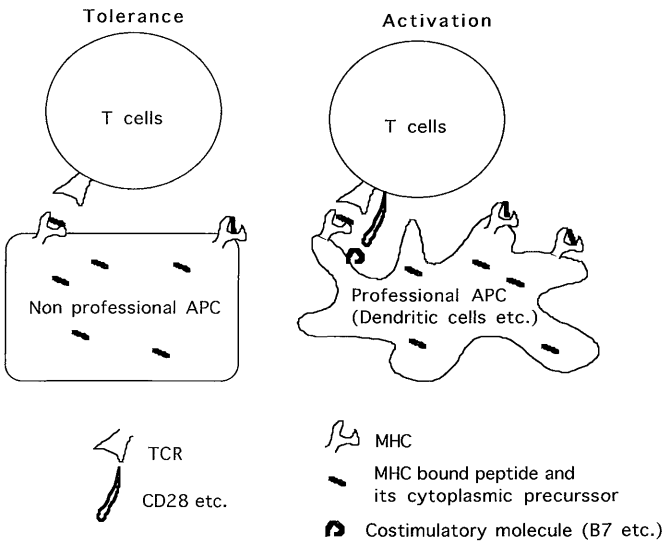

Fig. 1
Two signal model of T cell activation.
Two signals are required for full T cell activation. First signal is delivered by the occupancy of TCR by MHC/peptide. Second signal, which is called costimulation, is delivered by the occupancy of CD28 by B7 molecules etc. Professional APC express these costimulatory molecules and can fully activate T cells. Non-professional APC lack the ability to deliver the second signal. Occupancy of TCR without delivery of the second signal is believed to lead inactivation of the T cells.

The most efficient professional APC are dendritic cells[2]. In fact, the only known function of dendritic cells is the presentation of antigen to T cells. Dendritic cells are derived from bone marrow and express high levels of both MHC class I and class II as well as adhesion and costimulatory molecules. Mature dendritic cells from murine lymphoid organs can be identified by their expression of the integrin CD11c[3,4]. They include veiled cells in afferent lymphatics, interdigitating cells in the thymus, dermal dendrocytes, epidermal Langerhans cells as well as lymphoid dendritic cells in peripheral lymphoid tissue and blood. Recent studies revealed that dendritic cells are not homogeneous. They can be divided into two groups by their expression of CD8 molecule on cell surface[5,6]. The typical dendritic cells do not express CD8 and are myeloid origin, and is dependent on GM-CSF for growth. They collect antigens in the peripheral site, migrate to the secondary lymphoid organ, and activate specific T cells. They include dendritic cells of Langerhans and monocyte/macrophage lineages. Dendritic cells expressing CD8 have been identified recently. They derive from lymphoid-restricted progenitors[7] and are $CD8^{+}DEC\text{-}205^{+}CD11\beta^{-}$[5]. These cells are present in both thymus and lymph nodes, express Fas ligand, and can induce apoptosis in reactive T cells, suggesting that these dendritic cells might have regulatory function[8]. These cells are not dependent on GM-CSF[9].

Antigen expressed in non-professional APC

Naive T cells, which migrate to the periphery out of thymus, recirculate through lymphatics and blood (Fig. 2)[10,11]. They are activated when they recognize antigenic peptide/MHC presented by professional APC in the secondary lymphoid organs. Thus, peripheral antigens have to be carried to the draining lymph nodes to prime T cells. Microbes invading inside the skin, for

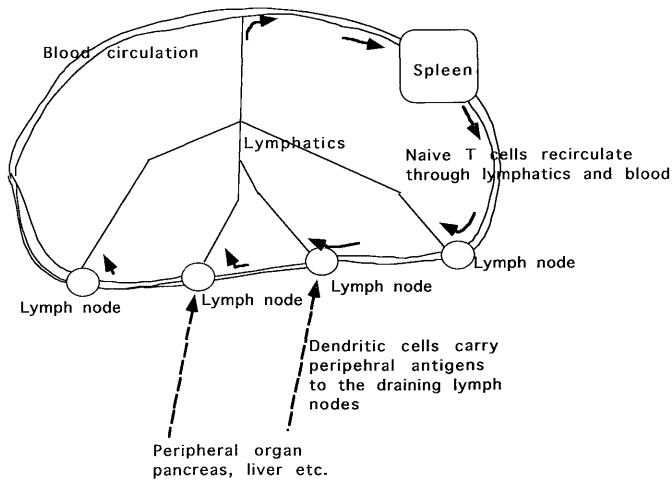

Fig. 2
Recirculation of naive T cells.
Naive T cells recirculate through lymphatics and blood, and do not migrate to the periphery. Peripheral antigens have to be carried to the draining lymph nodes to prime specific naive T cells.

example, will be taken up by Langerhans' cells, which migrate to the draining lymph nodes, where they activate specific T cells. In general, macrophages or immature dendritic cells have phagocytic function. They take up peripheral antigens by phagocytosis or endocytosis and present them to T cells in association with MHC class II molecules. How are antigens derived from microbes that invade intracellular compartment presented to T cells? When professional APC are infected by virus, they can present viral peptide derived from the cytoplasm in the context of MHC class I, and activate specific CD8 T cells (Fig. 1). However, not every intracellular microbe can infect professional APC. Many microbes are tissue tropic and infect only a particular tissue, which is often of non-hematopoietic origin. For example, hepatitis virus or liver-stage malaria only infects hepatocytes while influenza virus infects respiratory epithelium. They do not infect professional APC, but specific cytotoxic T cell response is induced in the infected individual. How do pathogen-derived antigens have access to MHC class I antigen presentation pathway, and activate naive CD8 T cells? Recent evidence indicates that these cells do not directly activate CD8 cells; instead, antigens derived from intracellular microbes are somehow taken up by professional APC and are presented in association with MHC class I molecules[12]. This type of indirect priming of T cells by professional APC is called cross-priming (Fig. 3).

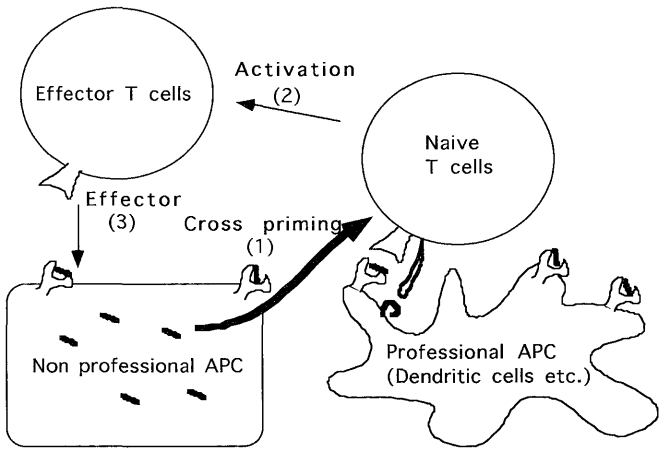

Fig. 3
Cross-priming.
Non-professional APC cannot directly activate naive T cells. Cytosolic antigens in non-professional APC, such as viral products, are somehow presented to naive T cells by MHC class I antigen presentation pathway of professional APC. Once activated, naive T cells differentiate into effector T cells, which can recognize MHC/peptide on non-professional APC.

The concept of cross-priming is not new. It was originally described more than 20 years ago when the ability of MHC-mismatched cells to prime minor histocompatibility antigen specific cytotoxic T cell responses was reported[13,14]. The term cross-priming is now more widely used to describe the priming of CD8 T cells by exogenous antigens. It appears that exogenous antigens are somehow internalized, digested by cytoplasmic proteasome, and transported into endoplasmic reticulum through Tap molecule where the antigenic peptides binds to MHC class I molecules[15]. Examples of cross-priming has been accumulating. In addition to the priming with minor histocompatibility antigens, cell-associated[16] or particu late antigens[17,18,19] are effective antigens for cross-priming. Antigens associates with cells undergoing apoptosis also can be taken up by APC and presented in the context of MHC class I molecules[20]. It was clearly demonstrated that cells of the hematopoietic lineage, most likely professional APC, mediate priming of the naive T cells against tissue-tropic virus[21]. Furthermore, presentation of exogenous antigen by class I pathway appear to take place not only for foreign antigens but also for self-antigens[22]. In transgenic mouse model in which membrane bound form of ovalbumin (OVA) was expressed in pancreatic β cells, specific T cells were activated by cross-priming of OVA by bone marrow derived APC.

The molecular basis of cross-priming is poorly understood. One of the candidate molecules, which may be involved in this process, is heat-shock protein. Heat-shock protein gp96 or hsp70 purified from tumor cells associate with tumor specific antigenic peptides and can induce specific cytotoxic T cell responses and protective immunity against the original cancer[23,24]. Similarly, heat shock protein gp96 was shown associated with dominant viral peptide[25], and also can prime minor histocompatibility antigen-specific T cells[26]. It is likely that heat shock protein derived from non-professional APC is involved in the process of cross-priming.

The second point that should be considered in priming naive T cells by antigens expressed in peripheral tissue is circulation of naive T cells (Fig. 2). Naive T cells recirculate through secondary lymphoid organs and do not migrate into peripheral tissue[10,11]. Therefore, naive T cells do not have opportunity to contact antigens expressed in peripheral tissue alone, unless they are transported into the secondary lymphoid organs. When the peripheral tissue is infected with microbes, the local environment is altered by the initial innate immune responses resulting in activation of dendritic cells, which migrate to the regional lymph nodes where they activate naive T cells (Fig. 2). This migration of dendritic cells, however, may occur constantly without local inflammation. This possibility was suggested in an experimental

model system using transgenic mice[22]. OVA-specific CD8 cells from the TCR transgenic mice were injected into transgenic mice, which express a membrane-bound form of OVA in the pancreatic islet β cells and kidney. Transgenic T cells specifically accumulated in the draining lymph nodes of pancreas and kidney, and they showed activated phenotype indicating that tissue-associated neo-self antigen can be constantly presented by MHC class I pathway in the draining lymph nodes. Therefore, at least under certain conditions, not only pathogen-derived antigens but also self-antigens in non-lymphoid tissue can be presented to naive recirculating T cells.

Central Tolerance

The most important organ where self-reactivity of lymphocytes is controlled is the thymus, the very organ in which T cells are generated. This control is mainly performed by apoptosis of T cells expressing high affinity TCR for MHC/self-peptide and is called negative selection. Antigen encounter of lymphocytes at this stage results in death or anergy induction rather than activation. The deletion of self-reactive thymocytes was clearly shown by using TCR transgenic mouse models[27,28] and superantigen system[29].

The most efficient cells that are responsible for presenting antigen and inducing apoptosis in the thymus appear dendritic cells that are present in the cortico-medullary junction as well as thymic medulla[2]. The majority of dendritic cells in the thymus are lymphoid dendritic cells identified by their expression of CD8. These cells express Fas ligand are suggested to be involved in regulatory function rather than activation of T cells[5], which appear appropriate.

Since developing thymocytes are designed to die when they encounter the relevant antigen in the thymus, any MHC bound peptide that are accessible to the thymus should be tolerogenic. The following antigen can be presented in the thymus resulting in negative selection of specific thymocytes.

(1) Antigens expressed in the cells of the thymus, which include thymic epithelium, dendritic cells and T cells. Antigenic peptides expressed in these cells may be presented in association with both MHC class I and class II, and are tolerogenic for developing CD4 and CD8 cells.

(2) Exogenous antigens that have access to the thymus can also induce negative selection. When the mice were chronically infected with lymphocytic choriomeningitis virus (LCMV) prior to birth, specific T cells were shown depleted[30].

(3) Some of the tissue specific antigens might leak into circulation, reaching developing thymocytes. Thymus-blood barrier appears to control the access of peripheral antigen into the thymus, although its precise mechanisms are not sufficiently understood.

Peripheral Tolerance

Why are peripheral mechanisms of tolerance necessary? The central tolerance ensures that T cells specific for peptides derived from ubiquitous self-protein are depleted. They can be presented by thymic APC to developing thymocytes inducing apoptosis, net result of which is negative selection of self-reactive T cells. However, not all self-peptides are available in the thymic APC. Each peripheral organ has its unique protein expression, and there should be protein expressed only in the periphery. (For immunologists, any organ other than thymus and bone marrow is peripheral organ.) Some of them might circulate through blood, reaching thymus, and induce central tolerance. However, proteins expressed in the cytoplasm or attached on cell surface may not be able to reach thymus. Therefore, T cells specific for such tissue specific antigens cannot be depleted in the thymus. They mature in the thymus and become naive T cells circulating in the lymphatics and blood. There should be mechanisms regulating these self-reactive T cells, since these T cells are potentially hazardous to self-tissue.

How the immune system avoids the attack of self-reactive T cells? Since induction of tolerance results in loss of function or cell death, it is difficult to study unless we identify relevant T cells. In this respect, study of peripheral tolerance has been greatly benefited by the use of transgenic mouse models (Fig. 4). TCR transgenic mice enabled us to follow the fate of specific T cells even after they are functionally inactivated (Table 1). Antigen transgenic mice were also developed in which artificial model antigen was expressed in a particular tissue under the control of tissue-specific promoter (Table 2). However, the results of these studies varied greatly. Several transgenic mouse models expressing a neo-self-antigen exclusively in a particular tissue retained normal reactivity to immunogenic forms of the same antigen. Thus, circulating T cells ignored the peripheral neo-self antigen. In other transgenic models, some form of tolerance was actively acquired. I will discuss some of the representative transgenic model systems.

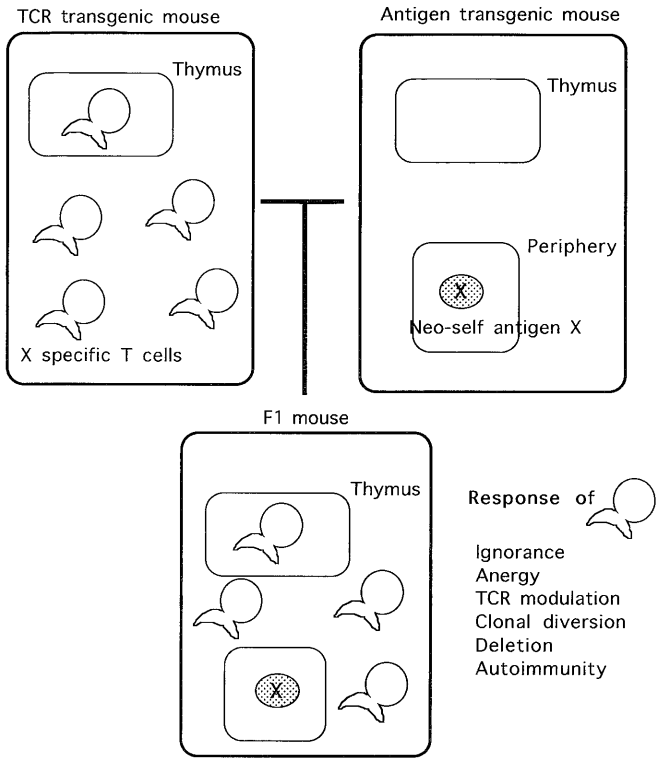

Fig. 4
Transgenic model of T cell tolerance.
The majority of T cells in TCR transgenic mice express the transgenic TCR on cell surface. The fate of these cells can be monitored using the TCR clonotype-specific antibody. Antigen transgenic mice express neo-self antigen X under the control of an appropriate promoter. Mice expressing the neo-self antigen in peripheral organs, and never in the thymus, are used for the study on the mechanisms of T cell tolerance to peripheral antigens. TCR transgenic and antigen transgenic mice are crossed to produce F1 mice in which the majority of T cells are specific for the neo-self antigen.

Table 1. TCR transgenic mice often used for the study of T cell immunology

Name	MHC restriction	Antigen	TCR	References
H-Y	D^b	H-Y	Vβ8.2	27)
2C	(allorective)	L^d	Vβ8.2	60)
P14	D^b	LCMV	Vα2Vβ8.1	30)
OT-I	K^d	$OVA_{257-264}$	Vα2Vβ5	61)
Des	(allorective)	K^b		42)
R28	A^k	RNase	Vα4Vβ6	62)
2B4	E^k	MCC_{87-103}	Vα11Vβ3	63) 64)
5C.C7	E^k	MCC_{87-103}	Vα11Vβ3	65)
DO11.10	A^d	$OVA_{323-339}$	Vβ8	66)
TCR-LACK	A^d	LACK	Vα8Vβ4	50)
3A9	K^k	HEL_{46-61}	Vα3Vβ8.2	67)

H-Y: male antigen
LCMV: lymphocyte choriomeningitis virus
OVA: ovalbumin
MCC: moth cytochrome C
LACK: Leishmania major LACK antigen
HEL: hen egg lysozyme

Table 2. Neo-self antigen transgenic mice

Name	Antigen	Promoter	Expression	References
CPR-K^b	K^b	CRP	liver	68)
RIP-K^b	K^b	RIP	pancreas	40)
GFAP-K^b	K^b	GFAP	neuroectodermal	42)
LCMV		RIP	pancreas β cells	31) 69)
RIP-Tag	SV40 T antigen	RIP	pancreas β cells	47)
RIP-OVA	OVA	RIP	pancreas β cells	22, 32)
RIP-I-E	E	RIP	pancreas β cells	38, 39)
INS-HEL	HEL	RIP	pancreas β cells	43) 48)
TLK-1, -2	HEL	rat thyroglobulin promoter	thyroid epithelium	48)

CRP: human complement reactive protein
RIP: rat insulin promoter
GFAP: glial fibrillary acidic protein

(1) Ignorance

Ohashi et al. generated transgenic mice, in which the lymphocyte choriomeningitis viral (LCMV) glycoprotein (GP) was expressed in the β islet cells of the pancreas under the control of rat insulin promoter[31]. The fate of self-reactive T cells was followed by breeding these transgenic mice with TCR transgenic mice whose transgenic receptor is specific for LCMV GP. Specific T cells in the double transgenic mice were not activated or depleted. They ignored the presence of neo-self antigen. However, when the mice were infected with LCMV, specific T cells were activated and attacked islet β cells resulting in $CD8^+$ cell-mediated diabetes. Similar observation was made in transgenic mice expressing ovalbumin in pancreatic β cells. When the mice were fed with ovalbumin, self-reactive T cells were activated, leading to the onset of autoimmune diabetes[32]. These studies indicate that naive T cells do not necessarily recognize self-proteins expressed in the periphery. It appears that they ignore peripheral antigen when the level of protein expression is low, or when appropriate co-stimulatory signal is not provided. However, once specific T cells are activated by the local infection or inflammation, they recognize those self-antigen, inducing autoimmune disease. This type of self-reactivity in the periphery may also occur against antigens expressed in the thymus[33]. In TCR Vβ8.1 transgenic mice, some of the self-superantigen specific T cells escape thymic negative selection and become mature peripheral T cells. These T cells become auto-reactive when activated by environmental stimuli[34,35]. This type of autoreactivity is observed probably because the threshold of thymic negative selection is lower than that of re-activation of previously activated peripheral T cells[35].

(2) Anergy

T cell anergy can be induced in mature T cells *in vivo* by inoculation of superantigen[36,37]. It also occurs in T cells reactive to tissue specific antigens as demonstrated in some transgenic mouse models. In the transgenic

mice in which MHC class II I-E was expressed in pancreatic β cells, specific T cells were not depleted but were unresponsive to the TCR stimulation[38,39]. CD8 cytotoxic T cells also can become unresponsive to TCR occupancy. When transgenic mice expressing allogenic MHC class I K^b in islet β cells were crossed with TCR transgenic mice expressing K^b-specific TCR, peripheral CD8 T cells became unresponsive to antigenic stimulation and the mice did not develop autoimmune diseas e[40,41]. When these transgenic mice were crossed with transgenic mice expressing interleukin 2 in β cells of the pancreas, the triple transgenic mice developed diabetes. It is not clear, whether anergy is an established state of long lasting peripheral tolerance or is a transient state of tolerant T cells undergoing apoptosis.

(3) TCR modulation

In a transgenic mouse model, MHC class I K^b was expressed under the control of the glial fibrillary acidic protein promoter in cells of neuroectodermal origin outside the thymus[42]. When these mice were crossed with transgenic mice expressing K^b specific TCR, specific T cells disappeared from the periphery. These cells, however, reappeared in culture, suggesting that these self-specific T cells down regulated TCR and CD8 molecules *in vivo*. This mechanism was proposed as one of peripheral tolerance, but it is unclear how much this type of tolerance contributes self-tolerance in normal animals. It is not common that cells, which do not express TCR *in vivo*, gain its expression *in vitro*.

(4) Clonal diversion

Transgenic mice expressing an influenza hemagglutinin (HA) on islet β cells were crossed with TCR transgenic mice specific for a MHC class II-restricted HA peptide[43]. Double transgenic mice showed either resistance or susceptibility to spontaneous diabetes depending on the genetic background, which was not linked to MHC. Functional studies on autoreactive CD4 T cells from resistant mice showed that they were predisposed toward differentiation to Th2 phenotype, which is not pathogenic. These CD4 cells from the resistant double transgenic mice showed evidence of prior activation by antigen, suggesting that the disease might be actively suppressed by autoreactive Th2 cells. Therefore, functional deviation of CD4 cell differentiation appears also a mechanism to avoid autoimmune diseases in the case of antigen presented by MHC class II.

(5) Deletion

Clonal deletion of T cells also is one of the mechanisms of peripheral T cell tolerance. Inoculation of superantigen into mice *in vivo* induces specific T cell proliferation followed by deletion[44,45]. Also, cross-presentation of neo-self-antigens can induce peripheral T cell tolerance by deletion of autoreactive CD8 T cells[22]. CD95 (Fas) appear to be involved in the deletion of autoreactive CD8 T cells in the secondary lymphoid organs in this system[46].

Autoimmunity

In the transgenic model systems, there were several cases in which self-tolerance was not established. I will discuss factors that might be involved in the pathogenesis of autoimmune diseases in these model systems.

(1) Number of autoreactive T cells

When there were too many autoreactive T cells, self-tolerance could not be maintained[47]. SV40 T antigen-specific TCR transgenic mice expressing the transgenic TCR on either 10% or 90% of peripheral T cells were crossed to the transgenic mice expressing the antigen in pancreatic β cells. Mice expressing the transgenic TCR on 10% of T cells showed partial deletion of SV40-specific T cells in the periphery and unresponsiveness of those that remained. In contrast, crossbred mice in which transgenic T cells comprised a majority of T cell population were not tolerant.

(2) Local concentration of self-antigen

The concentration of self-antigen in a discrete organ also affects the responsiveness of the antigen-specific peripheral T cells[48]. Hen egg lysozyme (HEL) was expressed on the thyroid epithelium, pancreatic β-cells or systematically using rat throglobulin promoter, insulin promoter or MHC class I promoter, respectively. These mice were crossed with TCR transgenic mice whose TCR is specific for HEL, and the development of tolerance/autoimmunity was investigated. Autoreactive T cells were present in the periphery and initiated autoimmune inflammation when self-antigen was concentrated in the thyroid or pancreas.

(3) Local environment

Local environment also affects the etiology of autoimmune disease. In transgenic mouse models in which neo-self antigen was expressed in pancreatic β cells, the tolerant state was broken resulting in autoimmune disease when IL-2[41] or IFN-γ [49] was co-expressed in the target organ. These cytokines may directly or indirectly influence activation state of the specific T cells. It is also likely, that local APC were activated by these cytokines resulting in priming of the specific T cells in the draining

lymph nodes. Alternatively, local cytokine environment can down-regulate autoimmune attack by T cells. Transgenic mice expressing TNF-α and *Leishmania major* LACK antigen in the pancreatic β cells exhibited impaired ability to mount a T cell response against LACK, suggesting modulation of the self-reactive T cell response by this cytokine[50]. Recently, the involvement of CD30 in the regulation of autoreactive T cells in the periphery was demonstrated[51]. In this model, OVA specific CD8 T cells from CD30 knockout mice were transferred into transgenic mice expressing OVA in pancreatic islet β cells. They were much more auto-aggressive than wild-type cells, leading to the complete destruction of pancreatic islets and the rapid onset of diabetes. CD30 signaling appears to limit the aggregation potential of CD8 effector T cells, and protects the organ from autoimmune attack.

Self-tolerance vs. host defense

Microbes that infect only non-hematopoietic tissue cannot directly activate naive CD8 T cells. The antigen derived from such microbes has to be taken up by professional APC and cross-presented to CD8 cells (Fig. 5). Likewise, self-antigens expressed in the peripheral tissue can be presented by professional APC under certain conditions[22]. If so, how immune system distinguishes pathogens and self-antigens to which T cells should be primed and tolerized, respectively? The answer is not completely clear, but several possibilities can be considered.

(1) Self-antigens are presented continuously while antigens derived from pathogens are presented only during the infection. T cells are tolerized when antigen is continuously presented by APC ever since the immune system is established, while they are activated when antigen become available transiently later in life. For some antigens which are present in the periphery at relatively high concentrations, self-antigens appear continuously presented by dendritic cells in the draining lymph nodes leading to apoptosis of reactive T cells. However, not all self-antigens may be continuously presented. Also, some self-proteins may be expressed later in life or their expression may fluctuate during the life of the individual.

(2) Dendritic cells, the most prominent professional APC, need to be activated in order to stimulate naive CD8 cells (Fig. 5). This activation signal comes from CD4 helper cells via CD40-CD40 ligand interaction, cytokine TNF-α or LPS derived from gram-negative

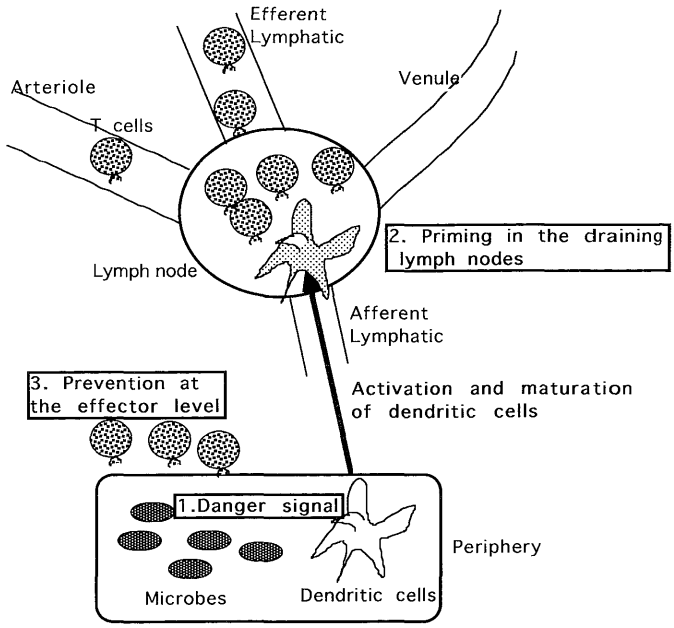

Fig. 5
Multiple steps in T cell activation vs. inactivation
TNF-α, CD40 ligand, or products of microbes (danger signal) activate dendritic cells in the peripheral tissue. Activated dendritic cells carry antigens to the draining lymph nodes where they activate specific T cells. Activated T cells differentiate to effector cells and migrate to the periphery where they exert their effector function. The immune response is regulated in each step of this pathway.

bacteria[52,53,54]. In the absence of this signal, dendritic cells cannot activate naive CD8 cells. After pathogen infect peripheral tissue, the initial host defense mediated by macrophages or granulocytes results in local production of cytokines including TNF-α, and activation of local APC. The product of microbes themselves, such as LPS, can also activate dendritic cells[2]. The dendritic cells activated by this local environment carry pathogen-derived antigens to the draining lymph nodes where they activate specific T cells. This environment, which activates dendritic cells, was termed "danger signal" by Polly Matzinger[55]. In contrast, dendritic cells, which derive from non-inflammatory tissue, are resting cells, and may not be able to activate T cells in the draining lymph nodes.

The "danger signal" may also be delivered by the local stress responses. Heat shock proteins up regulated by stress responses may stimulate T cells expressing $\gamma\delta$ T cell receptor[56] or monocytes[57] resulting in cytokine production. Finally, it should be noted that the regulation of this danger signal is not simple. IL-10 has been shown to convert dendritic cells into tolerance inducing form[58]. Although physiological role of this effect is unclear, it is conceivable that the activity

of dendritic cells can be regulated both positive and negative directions depending on the local environment.

(3) There are regulatory T cells which suppress activity of self-reactive T cells in the peripheral T cell pool. They are $CD25^+CD4^+$ subset of mature T cells, which constitute 5-10% of peripheral $CD4^+$ T cells in mice[59]. Elimination of this population leads to spontaneous development of various tissue specific autoimmune diseases. The effector phase of these regulatory T cells appears non-specific. It is unclear how these regulatory T cells relate to the mechanisms of specific T cell activation/inactivation described in this review.

Summary

Recent advances in immunology have uncovered varieties of mechanisms underlying self-tolerance. They include clonal deletion, clonal anergy, ignorance, receptor modulation, and clonal diversion. These mechanisms are manifested at several levels during T cell maturation and activation. First and perhaps the most important step is thymic negative selection, where developing thymocytes that encounter any antigen are specifically tolerized. Self-reactive T cells that escape this selection has to undergo peripheral mechanisms of tolerance (Fig. 5). Here, the second level of T cell tolerance occurs in the secondary lymphoid organs. Since naive T cells recirculate through lymphatics and blood, peripheral antigen has to be taken up by local APC and carried to the draining lymph nodes to inactivate specific T cells. If self-reactive T cells are activated, they migrate to the tissue where the antigen is expressed. The third level of resistance to autoimmunity appear to present at the target tissue, which can be broken down by the local cytokine environment. In addition to these mechanisms, T cells of $CD4^+CD25^+$ phenotype suppress organ specific autoimmune diseases by mechanisms yet to be known.

Peripheral mechanisms of self-tolerance occur at multiple steps, which are similar to the induction of immune responses. How does immune system distinguish self-antigen to which response has to be avoided and foreign antigen to which it should attack? The precise mechanisms have not been elucidated, but "danger model" is an attractive idea. In this way, the mechanisms of immune system, which distinguishes self- vs. foreign-antigen, are expressed not at the level of single cell type, but are operationally manifested in a whole immune system. It is a complex system being regulated at multiple levels, the elucidation of which is attractive and challenging. Finally, understanding of these mechanisms is crucial for the improved care of patients of immune-related diseases as well as the development of novel vaccines. Improvement of the protective immunity is important for treatment of patients with infectious diseases and cancer, while down regulation of the specific immunity is crucial for patients of transplantation, autoimmune diseases, and allergic responses.

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