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Review

Uncovering the Differences between T Cell Tolerance and Immunity

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Abstract. In the last two decades, T cell function has been analyzed *in vitro* from many different angles, with a great deal of attention dedicated to the basic requirements of activation. During this time, a compendium of information has been collected and has proven to be invaluable. Paradoxically, very little is known about T cell activation and function *in vivo*. In the last decade, a number of models have been developed which allow the tracking of Ag-activated T cells *in vivo* and these studies have been instrumental in advancing the field of T cell biology. In particular, a new and emerging paradigm of T cell immunity is evolving.

Key words: T cell activation; immunity; immune memory.

Defining Activation, Tolerance and Memory

The larger part of this review is dedicated to examining CD4 T cell tolerance versus CD4 T cell activation. Activation is a somewhat confusing word as we do not define activation in the same way for each circumstance. For example, one who studies signal transduction may define activation by the ligation of a receptor, while a molecular biologist may consider the induction of gene expression as activation. Further, a cell biologist may regard proliferation or protein secretion as another form of activation. Depending on the subdiscipline of immunology being discussed, all of these definitions of activation may be applicable. Nevertheless, in the context of immunobiology, none of them may be accurate. Therefore, for the sake of clarity, I will deal with this issue by using the term "immunity", which translates to development of memory.

It seems that defining memory is much easier than defining tolerance. Memory is the ability of the immune system to recall a response against an antigen (Ag). In simple terms, being immune is equated with memory resulting from a previous exposure to an Ag. For example, one is immune to a previously injected Ag but is not usually able to mount an anamnestic response to an Ag to which the subject has never been exposed. Normally, a memory response is more vigorous than the initial response. The complexity of this issue is confounded when one begins to ask whether there are varying degrees of memory and why all memory responses are not equivalent. For example, why are certain vaccine strategies more effective than others? This latter point may be related to tolerance induction.

T cell tolerance to Ag is believed to occur in at least two sites in the body: the thymus (central tolerance) and the periphery (peripheral tolerance) [38, 56]. One other site is in the gut[71]. Tolerance in the thymus occurs during T cell development. As T cells differentiate into mature cells, they are screened for their ability to bind to peptides presented by major histocompatibility complex

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(MHC) molecules. The details of this process are not the focal point of this review and, therefore, I will only touch on the subject. Briefly, as T cells become double positive ($CD4^+$ $CD8^+$) expressing cells, they begin to survey MHC/peptide complexes and, if the affinity of the T cell receptor (TCR) for its ligand reaches beyond a certain threshold, the cell will then be programmed to die via apoptosis[3]. Negative selection also takes place in the bone marrow to eliminate self-reactive B cells.

Peripheral tolerance appears to be a more complicated process. Affinity of the TCR for MHC/peptide may play a role, but costimulatory molecules are also intimately involved, thereby complicating the situation even further. Similarly, understanding the end result of tolerance induction becomes a challenge because the readout of tolerance is hard to quantify. In the case of memory it is very clear what one looks for, namely, a recall response that can be measured by cell proliferation, cytokine production or target cell lysis. In the case of tolerance there should be little response to measure since the T cell is tolerant to the Ag. This raises several important issues: are T cells present but unresponsive (i.e. anergy), have they migrated to other parts of the body and, therefore, are unable to interact with the Ag, or are they dead? It may be that tolerance is a result of all of these processes to varying degrees. For example, it makes sense that a cell which is on its way to death will have little ability to produce cytokines or proliferate. In this case the cell would appear anergic but is simply moribund.

Models of T Cell Tolerance

There are several models of T cell tolerance that have allowed investigators to distinguish between these possibilities. The one commonality between each of these models is the ability to track Ag-activated T cells. This is critical, because it allows one to determine whether the cell population in question is present or not. Initial studies came from the use of superAg's (SAg)[39]. Early on it was noticed that T cells would be eliminated in the thymus of mouse strains which expressed mls proteins reactive to those T cells[46]. Later, soluble bacterial SAg was used to induce tolerance in mice. This later model has led to a number of important discoveries. For example, the use of SAg facilitated the discovery of T cell negative selection in the thymus[26]. Additionally, in the periphery it was found that SAg activation induced T cell death through an apoptotic mechanism[28]. SAg's have also been implicated in a vast number of human afflictions[4, 31]. Two of the foremost examples are food poisoning and toxic shock syndrome.

The SAg model is invaluable as a system to study peripheral tolerance. The key feature of the system is the ability to follow SAg-activated T cells over time based on the TCR Vβ chain that they bear. Therefore, monoclonal antibodies (mAb) specific to TCR Vβ chains can be used to follow activated T cells directly *ex vivo* through the use of a flow cytometer. It is widely known that as T cells are stimulated by SAg they will respond by blasting, producing cytokines and proliferating. Moreover, *in vivo* data has shown that SAg-specific T cells (both CD4- and CD8-bearing) will respond to SAg by clonally expanding, such that the numbers of SAg-reactive T cells are significantly higher than in uninjected mice[39]. Expansion peaks by 2 to 3 days after SAg injection and is followed by mass deletion of the responding cells. Deletion is usually completed by one week after injection and the vast majority of these cells die by apoptosis[28]. Notwithstanding, it has been shown that these cells can also become anergic[6, 27].

A second model developed by the Jenkins laboratory utilizes transgenic TCR-expressing T cells[29]. These cells are adoptively transferred into genetically similar wild-type mice such that the proportion of CD4 TCR transgenic T cells is approximately 1%. This low percentage of Ag-reactive T cells mimics physiological conditions and allows the T cells to be tracked after responding to the Ag. After T cell transfer, the mouse is injected with Ag and the T cells can then be monitored using an anti-idiotypic TCR mAb. The results of the SAg model and this model are generally very similar. Models which utilize transgenic TCR CD8 T cells specific to viral peptides have also been very useful[74].

Before the use of these models, it was impossible to determine the fate of the Ag-responsive cells *in vivo*. Earlier systems compared the physical state of the Ag more than the fate of T and B cell functions. In a set of pinnacle studies in the early 1960's by DRESSER[9, 10], it was shown that small amounts of bovine γ globulin were tolerogenic in mice compared to larger doses. At the same time, DRESSER noted that high-speed spins, which removed aggregated proteins, were essential for tolerogenicity. Even earlier studies by FREUND[13] showed the positive effects of various adjuvant oils on antibody synthesis. Paradoxically, it is still not clear how adjuvants specifically function to boost immunity. Taken together, the physical state of the Ag and the form of presentation can determine the quantity and quality of a lymphocyte response.

How Costimulatory Signals Affect T Cell Tolerance and Immunity

Differential signaling for tolerance induction over immunity was first proposed by BRETSCHER and COHN in 1970[5]. Specifically, they proposed that binding of one determinant by a surface antibody receptor alone would lead to tolerance, but this signal in conjunction with a carrier antibody molecule binding a second determinant would lead to antibody induction. Later, LAFFERTY and CUNNINGHAM modified this idea[34]. They proposed that two signals were needed to drive alloreactions. Signal one, Ag recognition (this is the same as the Bretscher and Cohn model) and signal two, or the costimulatory signal, are derived from the antigen presenting cell (APC). Some years later this model was confirmed with a series of *in vivo* and *in vitro* experiments performed by the Schwartz laboratory (see below).

Chemical modification of APC by treatment with the crosslinker 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide was shown to induce Ag-specific unresponsiveness in mice[25]. The results suggested that signal one without signal two induced tolerance in the Ag-activated lymphocyte population. Modified APC, in contrast to unmanipulated APC, were able to deliver signal 1 but were unable to deliver signal 2. A few years later a mAb was produced against murine CD28 and definitive evidence for a second signal was established[19]. CD28 is expressed on T cells at all times and is expressed on immature T cells in the thymus[17]. The ligand for CD28 is B7-1 (CD80) and B7-2 (CD86), which are expressed on APC[20]. In general, dendritic cells constitutively express these B7 molecules, but all other APC need to become activated in order for B7 expression to occur.

In the presence of signal 1 and signal 2, naïve T cells will increase stability of IL-2 mRNA, synthesize IL-2, increase expression of the IL-2R and then begin to clonally expand[24, 52]. This is the essence of the two-signal hypothesis. Effector T cells, on the other hand, do not seem to be as dependent on CD28 costimulation[8]. They are capable of secreting large quantities of cytokines without costimulation. Memory T cells also seem to be dependent on costimulation, but perhaps to a lesser degree than to naïve T cells[11]. Thus, during the differentiation of T cells after signals 1 and 2 multiple signaling pathways must be rewired to allow effector cell activation without costimulation. Currently it is not clear whether CD4 T cell effectors develop into memory cells or whether memory cells develop from a totally different cell lineage.

When naïve T cells are stimulated in the presence of signal 1 but in the absence of signal 2, these cells will become anergic. Anergy means the T cells do not proliferate and fail to make IL-2. Anergic T cell clones, however, produce other cytokines such as IFN-γ and IL-3 when stimulated[23]. In general, Th2 cells can also be induced to become anergic[72]. In doing so they will fail to provide help to B cells and be unable to proliferate. Thus, the two-signal model has allowed immunologists to compartmentalize T cell responses in a logical and manageable fashion.

Several confusing issues remain with regard to the two-signal hypothesis. In theory, constitutive B7 expression on circulating or localized APC should dramatically increase the likelihood of autoimmunity. In an elegant set of *in vivo* experiments, it was shown that constitutive B7 expression within the pancreas did not induce diabetes unless these transgenic mice were crossed with TNF-α transgenic mice which secrete[18]. Thus, signal one (self-pancreatic peptides) and signal two (transgenic CD80) were not sufficient to stimulate autoimmunity and, therefore, the two-signal model may be an oversimplification for long-lasting T cell activation *in vivo*. In a related set of experiments, it was shown that $CD28^{-/-}$ mice can clear certain viral infections and are able to generate protective responses to parasites even though these mice are less capable of generating memory T cells[54, 67]. These data suggest several important hypotheses and notions: 1) since two signals are not enough to stimulate autoimmunity, it is likely that other signals are important; 2) there must be other costimulatory signals which are sufficient to function in the absence of CD28 and the question is whether they function when CD28 is present. Taken together, we can conclude that two signals are sufficient to "activate" naïve T cells *in vitro* and *in vivo*, but T cell immunity *in vivo* is likely to involve multiple signals. Additionally, the difference between autoimmunity and tolerance induction is more complicated than competition for signal 2 and likely involves the action of proinflammatory cytokines such as TNF-α.

CD40 is a member of the TNF/NGF superfamily of receptors. CD40 is expressed on all APC, amongst other cell types, and its ligand CD154 (formally known as gp39 or CD40-ligand) is expressed on activated T cells[16]. CD154 binding to surface CD40 on APC enhances expression of CD80 and CD86[30, 53]. In turn, these APC are able to provide T cells with costimulation. Specifically, B cell surface Ig binding to its specific target (B cell signal 1) is not sufficient to induce expression of costimulatory molecules and, therefore, will induce anergy unless given signal 2. CD154 bear-

ing T cells can interact with resting B cells, which express a peptide/MHC complex that the T cell can recognize. CD154 will bind to CD40 (B cell signal 2), thereby causing up-regulation of CD80 and CD86. This will promote B cell activation and further T cell activation. How does the original T cell become activated so that it expresses CD154? Most likely this is through an activated macrophage or dendritic cell, which are probably activated nonspecifically by pathogens or adjuvants.

Although the CD40/CD154 system explains how T and B cells can communicate with each other, several important questions remain: How much time is required for this interaction? How many hits between T and B cells are necessary for full activation? Can the degree of interaction lead to different functional outcomes for T and B cells? The answers to these questions and others will provide important clues towards our understanding of immunity.

Other members of the TNF/NGF superfamily of receptors are also very important during immunity. OX40 is a receptor that is expressed on Ag-stimulated T cells that shows structural homology to CD40[70]. *In vivo* experiments have indicated that OX40 is expressed predominantly on CD4 T cells and not significantly on CD8 T cells[50]. OX40-ligand is restricted in its expression, but is found on activated APC[57]. OX40-ligand stimulation on B cells induces differentiation and contributes to Ig synthesis. Also, stimulation of OX40-ligand on dendritic cells leads to enhanced secretion of proinflammatory cytokines[48]. Collectively, these results suggest that OX40/OX40-ligand stimulation will improve immune responses *in vivo*.

Several key features concerning OX40 are now well established. OX40 stimulation on activated T cells leads to enhanced proliferation[50]. This result is also evident when Th1 and Th2 cells are examined, showing that there is no bias toward one population over the other[70]. This is supportive of *in vivo* data showing that OX40 stimulation can promote Th2 responses[12]. Additionally, it has been shown that OX40 and CD28 engagement on naïve T cells synergistically promote proliferation and IL-2 production[14]. Therefore, OX40 operates in a similar fashion to CD28 as a potent costimulatory signal for T cells.

4-1BB is another member of this family that shares a few structural and functional similarities to the other members[68]. In contrast to OX40, 4-1BB has been characterized as an activation molecule that is expressed on CD4 and CD8 T cells[51]. 4-1BB stimulation in mice carrying tumors leads to enhanced tumor regression[45]. In these mice, CD8 T cells possessed higher cytotoxic T lymphocyte (CTL) activity and exhibited a more robust cytokine response[55].

In our studies we found that 4-1BB was indeed expressed on both CD4 and CD8 staphylococcal enterotoxin A (SEA)-activated T cells, but the CD8 T cells expressed more of this molecule and enhanced 4-1BB expression was seen much earlier after Ag-stimulation[59]. When mice were injected with both SEA and an agonist mAb specific to 4-1BB, we found that proliferation was enhanced in the presence of the mAb. This is similar to what is observed by stimulating OX40 or CD28 on SEA-activated T cells *in vivo*. In contrast, however, 4-1BB stimulation led to a significant block in peripheral deletion, whereas the other two did not. Oddly enough, we found that the CD8 T cells were protected from deletion, but the CD4 T cells were still deleted. One possibility to explain this data is that 4-1BB is wired differently on CD8 versus CD4 T cells. Future experiments on the subject of comparing 4-1BB signaling between CD4 and CD8 T cells should be very interesting.

The Influence of Cytokines on T Cell Survival and Memory

The existence of T cell memory has been recognized for a number of years[11]. For much of this time there has been an ongoing debate concerning the importance of Ag in maintaining memory. For the most part this debate continues, but for CD8 T cells there seems to be little doubt that Ag is not essential[22, 35, 47]. For CD4 T cells it is still unclear as to the role of Ag, however, earlier reports show that memory T cells can survive in the absence of Ag[15]. Nevertheless, this contradicts previous studies, and so the debate continues[58].

The process of memory T cell generation is not well understood and represents one of the last great frontiers in basic immunology. Part of the reason this has been difficult to study is the lack of understanding of what constitutes memory. This has generated a wide spectrum of viewpoints and raises a variety of immunological questions, including: 1) are memory cells derived from effectors, 2) how many signals do T cells require to become memory cells, 3) how different are memory T cells from naïve cells, 4) where are memory cells located in the body and 5) how do memory cells avoid death signals? For the past decade only a few tenets have been established with regard to these and other questions[11]. First, memory cells are usually CD44hi, CD62-l negative and CD45RO positive. Secondly, memory T cells do seem to respond more vigorously to

stimuli than naïve T cells. And thirdly, recent data have shown that memory CD8 T cells are derived from effector cells and are not a separate lineage[49]. Although there may be examples when these tenets do not hold, in general they should be viewed as guidelines.

One of the more pressing issues is how effector cells are instructed to become memory cells. Recently, it has been shown that at least five cellular divisions are necessary for effector CTLs to develop into memory cells[49]. These data raise the question of what occurs during these divisions? Secondly, are these cells qualitatively different from the cells which have undergone <5 divisions, or have these cells out-competed the other cells for growth factors? These questions raise the issue of whether a subpopulation of effector cells possesses a selective advantage in obtaining a memory phenotype. For example, as T cells begin to clonally expand, it may be possible that a small proportion of effector cells receives a limiting signal(s) which grooms these cells to divide further and differentiate into memory cells. Potential signals which may drive this process are cytokines, accessory molecules on neighboring cells as they divide, or perhaps the lack of a death signal. Regardless of the outcome, these studies will lead to new and exciting concepts in immunology.

Another aspect of memory acquisition is the necessity of T cells to survive through the effector phase. It is widely believed that, as T cells progress to effector cells, a number of cell death mechanisms are activated. These include receptor-mediated death pathways such as Fas and trail, cytokine deprivation, and, possibly, steroid-induced death[32]. Regardless of the mechanism, effector T cells must thwart this attack in order to survive. Therefore, T cells have developed ways in which death can be circumvented.

Earlier it was noted by CHILLER and WEIGLE[7] that tolerance could be circumvented by administration of adjuvant. Specifically, they demonstrated that lipopolysaccharide (LPS) and soluble IgG induced immunity whereas soluble IgG alone induced tolerance. Some years later this study was expanded upon, using a SAg model which utilized SEA as the SAg[63]. Injection of SEA leads to profound deletion of the TCR Vβ3-reactive T cells[44]. If LPS is injected at the time of or one day after SEA injection, deletion is significantly inhibited. This was true for CD4 and CD8 T cells. Upon further examination, these rescued cells were found to be responsive to SEA and, therefore, were not anergic[65]. Mechanistically, it was shown that TNF-α was the critical factor in mediating rescue, since TNF-α neutralizing mAb inhibited LPS-induced rescue[63]. In an analogous experiment, injection of TNF-α and SEA led to rescue from deletion[64]. LPS seems to fit the description of a "danger" signal as proposed by MATZINGER[40, 41].

The irony of these results is that TNF-α is a well-noted death-inducing cytokine[73]. In a related scenario, it has been known for some time that the quintessential death receptor, Fas (CD95), can also be mitogenic when ligated[2]. Therefore, many factors must contribute to these types of differential responses, with microenvironment being no less important than any other. For example, cytokines may play a role in skewing one response over the other. This could be imagined in at least two ways. First, the quantity of certain cytokines in the microenvironment may promote survival over death. Large amounts of growth factors may block T cell apoptosis, whereas small amounts may not. A case in point is IL-2, which in large quantities will promote growth, but in small quantities will induce death via growth factor withdrawal[33, 36]. A second example suggests that the balance of various cytokines may influence survival over death. Exposure of activated T cells to proinflammatory cytokines may lead to one type of response, whereas anti-inflammatory cytokines may promote another. Additionally, there are certain cytokines, such as TGF-β, which have been characterized as having pleiotropic effects on cells, and TNF-α is no exception to this class of cytokines[69]. Thus, receptor activation driving a response in one circumstance and not in another is compatible with the idea that the outcome of an immune response is dependent upon multiple factors.

The mechanism of how TNF-α promotes T cell survival *in vivo* is unknown. Initial attempts were focused on analyzing a role for CD28 and B7. It was found that rescue was observed in mice treated with SEA/LPS and CTLA4-Ig as well as in $CD28^{-/-}$ mice[64]. These data strongly suggested that long-term survival was not driven by CD28 ligation. To investigate what role CD28 did play during SEA-induced proliferation, mice were given SEA and CTLA4-Ig and the fate of Vβ3 T cells was tracked. It was shown that the responsive cells did not grow to the same extent and deleted faster when CD28 ligation was blocked. One possibility to explain these data is that CD28 was not ligated efficiently. Therefore, experiments were conducted in which CD28 would be ligated maximally by activated APC *in vivo*. Injection of anti-CD40 led to dramatic increases of surface B7 expression[30, 53]. SEA and anti-CD40 led to massive increases in clonal expansion, but deletion was not inhibited[42]. Blocking CD28 from ligation in this model completely inhibited CD4 T cell clonal growth. Therefore, these data collectively show that signal 2 is a growth signal, but not a long-term survival signal *in vivo*.

These data led to the hypothesis that three signals may provide optimal *in vivo* immunity[43], these being Ag, costimulation and a survival signal. This notion is currently being tested and initial results are supportive of this idea. Nevertheless, one must remember that 4-1BB ligation induced clonal expansion and selective rescue of the CD8, but not CD4 T cells[59]. In this case only two signals were necessary; however, it is unknown whether more costimulation or survival stimuli would enhance rescue.

Identifying survival signals has been actively pursued by a number of laboratories in a variety of systems. Recent reports have shown that IL-6 is a potent inhibitor of the spontaneously induced death of naïve T cells[61]. Additionally, two members of the IL-2 family of cytokines, IL-4 and IL-7, can also block spontaneous death[66]. When T cells are activated and set up for death by growth factor withdrawal, IL-2, -4, -7 and -15 can all block death[1, 62]. Interestingly, IL-2 and IL-15 induce survival with proliferation and IL-4 and IL-7 induce survival without proliferation. This is especially true with resting T cells, where rescue from apoptosis by IL-4, -6 and -7 was not associated with proliferation. This is even more intriguing when one considers the fact that IL-2, -4, -7 and -15 utilize a common receptor sub-unit for signaling[37]. Therefore, survival can occur via proliferation and in the absence of proliferation. Taken together, survival signals come in at least two varieties: ones that promote proliferation and others that do not. The mechanism which promotes one mode of action over the other will clearly involve the untangling of various signaling pathways and gene activation.

Closing Thoughts

Understanding the differences that drive tolerance versus immunity go beyond an academic exercise. These data are invaluable in our pursuit for an understanding of autoimmune disease, tumor immunology, infectious disease and vaccine development. Developing techniques that inhibit T cell growth will most certainly play a role in controlling autoimmune disease. Conversely, assisting T cell growth and survival will improve vaccine development by converting weak vaccines into potent ones. The greatest challenge, however, will be to control Ag-specific T cell function and avoid affecting cells that are bystanders. For example, it would be very dangerous to enhance global T cell growth for vaccination purposes in a patient who is suffering from an autoimmune disease. There will be a necessity to target the appropriate cells while sparing potentially dangerous cells.

A key characteristic of vaccination and in the establishment of autoimmune disease is the association of inflammatory responses. In almost all circumstances of autoimmune disease there seems to be a chronic inflammatory reaction. At the same time many of these autoimmune diseases, including arthritis and multiple sclerosis, are indeed dependent on T cells[60]. It is likely that this relationship between inflammation and pathogenic T cell responses is not a coincidence. Based on the data reviewed here, it is likely that proinflammatory cytokines are playing a role in keeping T cells alive. In particular, TNF-α is directly associated with this phenomenon. Additionally, a number of pathogenic organisms are associated with certain autoimmune diseases[21]. It could be predicted that these organisms provide the ammunition to induce an inflammatory response, which in turn potently activates T cells and that some of these T cells may be autoreactive. In the context of an appropriate inflammatory reaction they can differentiate into long-lived autoreactive memory cells.

These ideas support a model of three signals being critical for the induction of optimal immunity. Naturally, the first is Ag, the second some type of growth signal such as that from a costimulatory molecule and the third a survival signal, which may be derived from a danger signal. It has been shown that immunity is heightened if T cells are responding in the presence of proinflammatory cytokines. These data lend credence to the three-signal hypothesis, since the same responses are not potent when the third signal is omitted. This theory does not state that this is the only way that immunity can be induced: it states that three signals will potentiate a long-term response. The end result is the accumulation of memory cells.

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References

1. AKBAR A, N., BORTHWICK N. J., WICKREMASINGHE R. G., PANAYOITIDIS P., PILLING D., BOFILL M., KRAJEWSKI S., REED J. C. and SALMON M. (1996): Interleukin-2 receptor common gamma-chain signaling cytokines regulate activated T cell apoptosis in response to growth factor withdrawal: selective induction of anti-apoptotic (bcl-2, bcl-xL) but not pro-apoptotic (bax, bcl-xS) gene expression. Eur. J. Immunol., **26**, 294–299.

2. ALDERSON M. R., ARMITAGE R. J., MARASKOVSKY E., TOUGH T. W., ROUX E., SCHOOLEY K., RAMSDELL F. and LYNCH D. H. (1993): Fas transduces activation signals in normal human T lymphocytes. J. Exp. Med., **178**, 2231–2235.
3. ANDERSON G., HARE K. J. and JENKINSON E. J. (1999): Positive selection of thymocytes: the long and winding road. Immunol. Today, **20**, 463–468.
4. BERNAL A., PROFT T., FRASER J. D. and POSNETT D. N. (1999): Superantigens in human disease. J. Clin. Immunol., **19**, 149–157.
5. BRETSCHER P. and COHN M. (1970): A theory of self-nonself discrimination. Science, **169**, 1042–1049.
6. CAULEY L. S., Cauley K. A., SHUB F., HUSTON G. and SWAIN S. L. (1997): Transferable anergy: superantigen treatment induces $CD4^+$ T cell tolerance that is reversible and requires $CD4^-$ $CD8^-$ cells and interferon gamma. J. Exp. Med., **186**, 71–81.
7. CHILLER J. M. and WEIGLE W. O. (1973): Termination of tolerance to human gamma globulin in mice by antigen and bacterial lipopolysaccharide (endotoxin). J. Exp. Med., **137**, 740–750.
8. CROFT M. and DUBEY C. (1997): Accessory molecule and costimulation requirements for CD4 T cell response. Crit. Rev. Immunol., **17**, 89–118.
9. DRESSER D. W. (1962): Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen. Immunology, **5**, 378–388.
10. DRESSER D. W. and GOWLAND G. (1964): Immunological paralysis induced in adult rabbits by small amounts of protein antigen. Nature, **203**, 733–736.
11. DUTTON R. W., BRADLEY L. M. and SWAIN S. L. (1998): T cell memory. Annu. Rev. Immunol., **16**, 201–223.
12. FLYNN S., TOELLNER K. M., RAYKUNDALIA C., GOODALL M. and LANE P. (1998): CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. J. Exp. Med., **188**, 297–304.
13. FREUND J. (1951): The effect of parafin oil and mycobacteria on antibody formation and sensitization. Am. J. Clin. Pathol., **21**, 645–656.
14. GRAMAGLIA I., WEINBERG A. D., LEMON M. and CROFT M. (1998): Ox-40 ligand: a potent costimulatory molecule for sustaining primary CD4 T cell responses. J. Immunol., **161**, 6510–6517.
15. GRAY D. and MATZINGER P. (1991): T cell memory is short-lived in the absence of antigen. J. Exp. Med., **174**, 969–974.
16. GREWAL I. S. and FLAVELL R. A. (1996): The role of CD40 ligand in costimulation and T-cell activation. Immunol. Rev., **153**, 85–106.
17. GROSS J. A., CALLAS E. and ALLISON J. P. (1992): Identification and distribution of the costimulatory receptor CD28 in the mouse. J. Immunol., **149**, 380–388.
18. GUERDER S., PICARELLA D. E., LINSLEY P. S. and FLAVELL R. A. (1994): Costimulator B7-1 confers antigen-presenting-cell function to parenchymal tissue and in conjunction with tumor necrosis factor alpha leads to autoimmunity in transgenic mice. Proc. Natl. Acad. Sci. USA, **91**, 5138–5142.
19. HARDING F. A., MCARTHUR J. G., GROSS J. A., RAULET D. H. and ALLISON J. P. (1992): CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. Nature, **356**, 607–609.
20. HARRIS N. L. and RONCHESE F. (1999): The role of B7 costimulation in T-cell immunity. Immunol. Cell Biol., **77**, 304–311.
21. HAUSMANN S. and WUCHERPFENNIG K. W. (1997): Activation of autoreactive T cells by peptides from human pathogens. Curr. Opin. Immunol., **9**, 831–838.
22. HOU S., HYLAND L., RYAN K. W., PORTNER A. and DOHERTY P. C. (1994): Virus-specific CD8+ T-cell memory determined by clonal burst size. Nature, **369**, 652–654.
23. JENKINS M. K., PARDOLL D. M., MITZUGUCHI J., CHUSED T. M. and SHWARTZ R. H. (1987): Molecular events in the induction of a nonresponsive state in interleukin 2-producing helper T-lymphocyte clones. Proc. Natl. Acad. Sci. USA, **84**, 5409–5413.
24. JENKINS M. K., PARDOLL D. M., MIZUGUCHI J., QUILL H. and SCHWARTZ R. H. (1987): T cell unresponsiveness *in vivo* and *in vitro*: Fine specificity of induction and molecular characterization of the unresponsive state. Immunol. Rev., **95**, 113–135.
25. JENKINS M. K. and SCHWARTZ R. H. (1987): Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness *in vitro* and *in vivo*. J. Exp. Med., **165**, 302–319.
26. KAPPLER J. W., ROEHM N. and MARRACK P. (1987): T cell tolerance by clonal elimination in the thymus. Cell, **49**, 273–280.
27. KAWABE Y. and OCHI A. (1990): Selective anergy of Vβ8+, CD4+ T cells in Staphylococcus enterotoxin-B primed mice. J. Exp. Med., **172**, 1065–1070.
28. KAWABE Y. and OCHI A. (1991): Programmed cell death and extrathymic reduction of Vbeta8+ CD4+ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. Nature, **349**, 245–248.
29. KEARNEY E. R., PAPE K. A., LOH D. Y. and JENKINS M. K. (1994): Visualization of peptide-specific T cell immunity and peripheral tolerance induction *in vivo*. Immunity, **1**, 327–339.
30. KIENER P. A., MORAN-DAVIS P., RANKIN B. M., WAHL A. F., ARUFFO A. and HOLLENBAUGH D. (1995): Stimulation of CD40 with purified soluble gp39 induces proinflammatory responses in human monocytes. J. Immunol., **155**, 4917–4925.
31. KOTZIN B. L., YEUNG D. Y. M., KAPPLER J. and MARRACK P. (1993): Superantigens and their potential role in human disease, **54**, 99–165.
32. KRAMMER P. H., BEHRMANN I., DANIEL P., DHEIN J. and DEBATIN K. (1994): Regulation of apoptosis in the immune system. Curr. Opin. Immunol., **6**, 279–289.
33. KURODA K., YAGI J., IMANISHI K., YAN X. -J., LI X. -Y., FUJIMAKI W., KATO H., MIYOSHI-AKIYAMA T., KUMAZAWA Y., ABE H. and UCHIYAMA T. (1996): Implantation of IL-2-containing osmotic pump prolongs the survival of superantigen-reactive T cells explanded in mice injected with bacterial superantigen. J. Immunol., **157**, 1422–1431.
34. LAFFERTY K. J. and CUNNINGHAM A. J. (1975): A new analysis of allogeneic interactions. Aust. J. Exp. Biol. Med. Sci., **53**, 27–42.
35. LAU L. L., JAMIESON B. D., SOMASUNDARAM T. and AHMED R. (1994): Cytotoxic T-cell memory without antigen. Nature, **369**, 648–652.
36. LENARDO M. J. (1991): Interleukin-2 programs mouse αβ T lymphocytes for apoptosis. Nature, **353**, 858–861.
37. LEONARD W. J., NOGUCHI M., RUSSELL S. M. and MCBRIDE O. W. (1994): The molecular basis of X-linked severe combined immunodeficiency: the role of the interleukin-2 receptor gamma chain as a common gamma chain, gamma c. Immunol. Rev., **138**, 61–86.
38. MARRACK P., HUGO P., MCCORMACK J. and KAPPLER J. (1993): Death and T cells. Immunol. Rev., **133**, 119–129.

39. MARRACK P. C. and KAPPLER J. W. (1990): The staphylococcal enterotoxins and their relatives. Science, **248**, 705–711.
40. MATZINGER P. (1994): Tolerance, danger, and the extended family. Annu. Rev. Immunol., **12**, 991–1045.
41. MATZINGER P. (1998): An innate sense of danger. Semin. Immunol., **10**, 399–415.
42. MAXWELL J. R., CAMPBELL J. D., KIM C. H. and VELLA A. T. (1999): CD40 activation boosts T cell immunity *in vivo* by enhancing T cell clonal expansion and delaying peripheral T cell deletion. J. Immunol., **162**, 2024–2034.
43. MAXWELL J. R., WEINBERG A., PRELL R. A. and VELLA A. T. (2000): Danger and OX40 receptor signaling synergize to enhance memory T cell survival by inhibiting peripheral deletion. J. Immunol., **164**, 107–112.
44. MCCORMACK J. E., CALLAHAN J. E., KAPPLER J. and MARRACK (1993): Profound deletion of mature T cells *in vivo* by chronic exposure to exogenous superantigen. J. Immunol., **150**, 3785–3792.
45. MELERO I., SHUFORD W. W., NEWBY S. A., ARUFFO A., LEDBETTER J. A., HELLSTROM K. E., MITTLER R. S. and CHEN L. (1997): Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat. Med., **3**, 682–685.
46. MORRISSEY P. J., BRADLEY D., SHARROW S. O. and SINGER A. (1983): T cell tolerance to non-H-2-encoded stimulatory alloantigens is induced intrathymically but not prethymically. J. Exp. Med., **158**, 365–377.
47. MULLBACHER A. (1994): The long-term maintenance of cytotoxic T cell memory does not require persistence of antigen. J. Exp. Med., **179**, 317–321.
48. OHSHIMA Y. et al. (1997): Expression and function of OX40 ligand on human dendritic cells. J. Immunol., **159**, 3838–3848.
49. OPFERMAN J. T., OBER B. T. and ASHTON-RICKARDT P. G. (1999): Linear differentiation of cytotoxic effectors into memory T lymphocytes. Science, **283**, 1745–1748.
50. PATERSON D. J., JEFFERIES W. A., GREEN J. R., BRANDON M. R., CORTHESY P., PULLAVES M. and WILLIAMS A. F. (1987): Antigens of activated rat T lymphocytes including a molecule of 50,000 Mr detected only on CD4 positive T blasts. Mol. Immunol., **24**, 1281–1290.
51. POLLOK K. E., KIM Y. -J., ZHOU Z., HURTADO J., KIM K. K., PICKARD R. T. and KWON B. S. (1993): Inducible T cell antigen 4-1BB. Analysis of expression and function. J. Immunol., **150**, 771–781.
52. POWELL J. D., RAGHEB J. A., KITAGAWA-SAKAKIDA S. and SCHWARTZ R. H. (1998): Molecular regulation of interleukin-2 expression by CD28 co-stimulation and anergy. Immunol. Rev., **165**, 287–300.
53. ROY M., ARUFFO A., LEDBETTER J., LINSLEY P., KEHRY M. (1995): Studies on the interdependence of gp39 and B7 expression and function during antigen-specific immune response. Eur. J. Immunol., **25**, 596–603.
54. SHAHINIAN A., PFEFFER K., LEE K. P., KÜNDIG T. M., KISHIHARA K., WAKEHAM A., KAWAI K., OHASHI P. M., THOMPSON C. B. and MAK T. W. (1993): Differential T cell costimulatoryr equirements in CD28-deficient mice. Science, **261**, 609–612.
55. SHUFORD W. W., KLUSSMAN K., TITCHLER D. D., LOO D. K., CHALUPNY J., SIADAK A. W., BROWN T. J., EMSWILER J., RAECHO H., LARSEN C. P., PEARSON T. C., LEDBETTER J. A., ARUFFO A. and MITTLER R. S. (1997): 4-1BB constimulatory signals preferentially induce $CD8^+$ T cell proliferation and lead to the amplification *in vivo* of cytotoxic T cell responses. J. Exp. Med., **186**, 47–55.
56. SPRENT J., LO D., GAO E. -K. and RON Y. (1998): T cell selection in the thymus. Immun. Rev., **101**, 173–190.
57. STUBER E., NEURATH M., CALDERHEAD D., FELL H. P. and STROBER W. (1995): Cross-linking of OX40 ligand, a member of the TNF/NGF cytokine family, induces proliferation and differentiation in murine splenic B cells. Immunity, **2**, 507–521.
58. SWAIN S. L. (1994): Generation and *in vivo* persistence of polarized Th1 and Th2 memory cells. Immunity, **1**, 543–552.
59. TAKAHASHI C., MITTLER R. S. and VELLA A. T. (1999): Cutting edge: 4-1BB is a bona fide CD8 T cell survival signal. J. Immunol., **162**, 5037–5040.
60. TANEJA V. and DAVID C. S. (1999): HLA class II transgenic mice as models of human diseases. Immunol. Rev., **169**, 67–79.
61. TEAGUE T. K., KAPPLER J. W., MARRACK P. and VELLA A. T. (1997): Interleukin-6 inhibits resting T cells from apoptosis. J. Immunol., **158**, 5791–5796.
62. VELLA A. T., DOW S., POTTER T. A., KAPPLER J. and MARRACK P. (1998): Cytokine-induced survival of activated T cells *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. USA, **95**, 3810–3815.
63. VELLA A. T., MCCORMACK J. E., LINSLEY P. S., KAPPLER J. W. and MARRACK P. (1995): Lipopolysaccharide interferes with the induction of peripheral T cell death. Immunity, **2**, 261–270.
64. VELLA A. T., MITCHELL T., GROTH B., LINSLEY P. S., GREEN J. M., THOMPSON C. B., KAPPLER J. W. and MARRACK P. (1997): CD28 engagement and proinflammatory cytokines contribute to T cell expansion and long-term survival *in vivo*. J. Immunol., **158**, 4714–4720.
65. VELLA A. T., SCHERER M. T., SCHULTZ L., KAPPLER J. W. and MARRACK P. (1996): B cells are not essential for peripheral T cell tolerance. Proc. Natl. Acad. Sci. USA, **93**, 951–955.
66. VELLA A., TEAGUE T. K., IHLE J., KAPPLER J. and MARRACK P. (1997): Interleukin 4 (IL-4) or IL-7 prevents the death of resting T cells: stat6 is probably not required for the effect of IL-4. J. Exp. Med., **186**, 325–330.
67. VILLEGAS E. N., ELLOSO M. M., REICHMANN G., PEACH R. and HUNTER C. A. (1999): Role of CD28 in the generation of effector and memory responses required for resistance to toxoplasma gondii. J. Immunol., **163**, 3344–3353.
68. VINAY D. S. and KWON B. S. (1998): Role of 4-1BB in immune responses. Semin. Immunol., **10**, 481–489.
69. WAHL S. M. (1994): Transforming growth factor beta: the good, the bad, and the ugly. J. Exp. Med., **180**, 1587–1590.
70. WEINBERG A. D., VELLA A. T. and CROFT M. (1998): OX-40: life beyond the effector T cell stage. Semin. Immunol., **10**, 471–480.
71. WEINER H. L. (1997): Oral tolerance for the treatment of autoimmune diseases. Annu. Rev. Med., **48**, 341–351.
72. YSSEL H., FASLER S., LAMB J. and DE VRIES J. E. (1994): Induction of non-responsiveness in human allergen-specific type 2 T helper cells. Curr. Opin. Immunol., **6**, 847–852.
73. ZHENG L., FISHER G., MILLER R. E., PESCHON J., LYNCH D. H. and LENARDO M. J. (1995): Induction of apoptosis in mature T cells by tumour necrosis factor. Nature, **377**, 348–351.
74. ZINKERNAGEL R. M., PLANZ O., EHL S., BATTEGAY M., ODERMATT B., KLENERMAN P. and HENGARTNER H. (1999): General and specific immunosuppression caused by antiviral T-cell responses. Immunol. Rev., **168**, 305–315.

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