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Molecular signals and genetic reprogramming in peripheral T-cell differentiation

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SUMMARY

Rearrangement of gene segments occurs in T lymphocytes during thymic development as the T-cell receptor (TCR) is first expressed, allowing T cells to become central regulators of antigen specificity in the acquired immune system. However, further development of T cells occurs after population of peripheral lymphoid tissues, which can result in T-cell expansion and differentiation into effectors of various immune function, or progression to memory T cells, anergic cells or death by apoptosis. This review focuses on more recent developments concerning the choices that peripheral T cells make between first encountering antigen through TCR recognition and death. These decisions are associated with a process of genetic reprogramming that alters the behaviour of cells so that immune responses are appropriately regulated.

INTERACTION OF T-CELL RECEPTOR AND COSTIMULATORY MOLECULE SIGNALLING IN ACTIVATION OF NAIVE T CELLS

Naive T cells have a high threshold for activation in order to prevent T-cell responses to self-antigens, particularly those which are not expressed in the thymus. This generally requires the antigen to be presented by a dendritic cell (DC). This contrasts with effector/memory cells that have lower thresholds of activation owing to their need to activate less efficient antigen-presenting cells (APCs), such as B cells and macrophages, in order to effect a response. DCs express particularly high levels of major histocompatibility complex (MHC) molecules, which will increase the likelihood of full T-cell activation after interaction with a T cell. However, it is the high and constitutive expression of costimulatory molecules that makes DCs so critical for the primary immune response. These costimulatory signals include those delivered by CD28 : CD80/CD86,[1] CD2 : CD58,[2] CD40 ligand (CD40L) : CD40,[3] lymphocyte function-associated antigen-1 (LFA-1) : intracellular adhesion molecule (ICAM),[4] OX40 : OX40L,[5] 4-1BB : 4-1BBL[6] and CD27 : CD70[7] interaction. Some have been shown to amplify the T-cell receptor (TCR)-induced signal, thus making a T-cell response possible with a limited number of MHC–peptide complexes. It has been proposed that CD28 costimulation reduces the number of TCR molecules that need to be engaged on a single T cell in order to induce activation, and amplifies TCR phosphorylation and consumption of Lck.[8] However, it also appears that CD28 and other costimulators, such as LFA-1, can transduce signals not induced by the TCR such as phosphatidyl inositol-3 (PI-3) kinase and C-JUN NH_2-terminal (JNK) mitogen-activated protein (MAP) kinase activation.[4,9,10]

The full complexity of the molecular events surrounding the engagement of the TCR with MHC–peptide complexes is only now becoming apparent. A host of costimulatory and signal-transducing molecules are recruited to membrane microdomains of TCR–MHC interaction known as immune synapses.[11–13] These areas involve rearrangement of the cytoskeleton to result in tight physical contacts;[14] signalling molecules then migrate in and out of the area in a highly coordinated fashion to deliver the complex patterns of biochemical events that are required for full activation.[15] The complexity of the process gives ample opportunity for regulation of signals, which may alter subsequent development of the T cell. Once the initial stimulus has been received, proliferation of the cells is the first response to be evoked. This allows the antigen-specific cells to be expanded so that there are sufficient numbers to deal with the situation. This expansion is the first stage of peripheral development and involves expression of cell-surface molecules such as CD25 and CD69 and utilization of interleukin (IL)-2 as an autocrine growth factor. These activated cells have been termed p T helper (pTh) cells as they are activated but have not differentiated to express full effector function, normally defined by their ability to produce high levels of effector cytokines such as interferon-γ (IFN-γ) and IL-4. Instead their cytokine-secreting potential is limited to IL-2 (required for proliferation) and possibly IL-3. The cells also become much more responsive to signals that will direct their future differentiation; for example they can respond to IL-4 alone by proliferating.[16] Activation of the JNK MAP kinase signalling pathway now becomes necessary for further T-cell development, although it is redundant for IL-2 secretion and proliferation.[17]

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Development of an effective primary T-cell response may require co-operation via cytokines between multiple responding T cells. This requirement could contribute effectively to peripheral tolerance as self-reactive naive T cells will encounter antigen individually as soon as they exit the thymus and become anergised or die as a result of lack of cytokine costimulation. In contrast, T cells responsive to foreign antigens will accumulate in the periphery and be activated together when antigen enters the body, resulting in an effective response.[18] Another mechanism of tolerance involves engagement of the cytotoxic T-lymphocyte antigen-4 (CTLA-4) (CD152) receptor instead of CD28 by CD80/CD86 on APCs.[19] CTLA-4 transduces an important signal that prevents tyrosine phosphorylation of TCR components[20] and its absence results in lymphoproliferative disease.[21] As well as blocking T-cell activation and IL-2 synthesis, it directly induces the secretion of transforming growth factor-β (TGF-β) (a potent immunosuppressive cytokine) by the T cell.[22] Unlike CD28, CTLA-4 expression is not constitutive and regulation of its expression may therefore be critical to tolerance induction.

SIGNALLING AND CYTOKINES IN THE GENERATION OF TYPE 1 VERSUS TYPE 2 EFFECTOR T CELLS

After T cells have received the full complement of activation signals, the decision between two major pathways of effector development is made. Differentiation into the type 1 and type 2 subsets of effectors expressing IFN-γ- or IL-4-associated cytokines, respectively, controls the balance of cell-mediated and humoral immunity, thus ensuring appropriate responses to pathogens. This occurs in both CD4 and CD8 subsets,[23,24] although important differences exist in the regulation of cytokine profiles between the two subsets,[25] and a fundamental functional dichotomy in the T cytotoxic (Tc) 1 and Tc2 subsets has yet to be demonstrated. Regulation of the development of type 1 versus type 2 CD4 and CD8 effector cells has traditionally been thought to be under the control of cytokines, primarily IL-4, IL-12 and IFN-γ,[26] derived from T cells or DCs/APCs, respectively.[27,28] These cytokine signals are transmitted through signalling via signal transducer and activator of transcription-4 (STAT-4),[29] (IL-12) and STAT-6[30] (IL-4). In humans, STAT-4 is also triggered by the IFN-α receptor so IFN-α can replace IL-12 in directing Th1 responses.[31] However, the primary stimulus that the cell receives is via the TCR and it is now apparent that differential signalling via the immune synapse can have a profound influence on type 1/type 2 development.[32] The intensity and duration of TCR triggering can clearly influence subsequent differentiation. This can be demonstrated by altering the dose of antigen used to trigger T cells. Low doses favour Th2 development while higher doses favour Th1 development.[33,34] As well as the number of TCRs triggered by peptide–MHC complexes, the affinity of the interaction also plays a critical role. Peptides that bind with high affinity to the TCR induce a predominant Th1 phenotype in subsequent effectors, while low-affinity peptides induce Th2 cells.[35–38] Affinity can be affected by altering the sequence of the peptide or the contact region of the TCR[39] and an analogous effect has been demonstrated in CD8 T cells.[40] The participation of the MHC in the interaction is also important as different MHC alleles can influence the Th1/Th2 balance because they affect affinity.[41] It has also been proposed that kinetic off-rates for dissociation of TCR–MHC complexes, rather than binding affinity itself, are more important for differential activation.[42] The duration of TCR engagement must also be considered because Th2 development requires a longer period of TCR engagement.[43] Together the data clearly indicate that the nature of the antigenic complex itself can determine the class of immune response initiated. This phenomenon is not restricted to models using altered peptide ligands – immunization of mice with different protein antigens can induce Tc1 or Tc0/Tc2 phenotypes in cytotoxic T lymphocytes (CTLs) *in vivo*.[44]

To explain how the affinity of cognate interactions and duration of signalling can alter development, a model is required in which qualitatively as well as quantitatively distinct signals can be transduced through the TCR complex.[45] Current data suggest that the balance of the three major TCR-transduced intracellular signals – protein kinase C (PKC), calcineurin and Ras/MAP kinase activation – can be regulated and result in altered type 1/type 2 differentiation. High levels of PKC activity combined with low calcium signals favour Th2 and Tc2 development, while a predominance of calcium signalling or MAP kinase activity favour type 1 development (ref. 40; A. Noble, unpublished). Peptides with weaker affinity for the TCR induce less phosphorylation of TCRζ chains[46] and greatly reduced calcium flux[47,48] while PKC signals appear to be relatively unchanged,[40] explaining their type 2-promoting effect. They are also more dependent on participation of the CD4 co-receptor to induce effective signalling.[49] Differences in the kinetics of signalling and association/dissociation of binding might result in net alterations in the balance of signals received after different time-periods of TCR ligation. Furthermore, interaction of TCR with the peptide–MHC complex may involve conformational changes that affect subsequent signalling events.[50] These findings extend the potential roles of altered peptides to every stage in T-cell differentiation because they are also able to induce positive selection during thymic development[51,52] and inactivation of differentiated effector T cells.[53] The immune synapse can therefore be thought of as the 'brains' of the T cell, controlling activities throughout life and able to transmit complex information from the outside world rather than simply acting as an 'on/off' switch. The major downstream targets of PKC, calcium and MAP kinase signalling are the nuclear factor (NF)-κB, nuclear factor of activated T cells (NFAT) and activator protein-1 (AP-1) transcription factors that initiate expression of various genes involved in T-cell activation. NFAT and AP-1 bind co-operatively to the IL-4 promoter region, and different NFAT family members can differentially regulate IL-4 expression.[54]

The mechanism of TCR control over effector differentiation may be mediated via altered cytokine synthesis.[55] Type 2-promoting TCR stimuli may increase the sensitivity of cells to the differentiative effects of IL-4 (A. Noble, unpublished) and the Th2-promoting effect of CD28 ligation is associated with increased phosphorylation of IL-4 receptor-α (IL-4Rα) and STAT-6 in the presence of IL-4, thus increasing IL-4 sensitivity without altering receptor expression.[56] Early secretion of IL-4 by activated T cells could down-regulate IL-12Rβ2 expression, inducing intermediate cells unable to enter the Th1 pathway.[57] Cytokines could regulate early IL-4 secretion; for example, IL-18 has recently been reported to enhance IL-4-dependent Th2

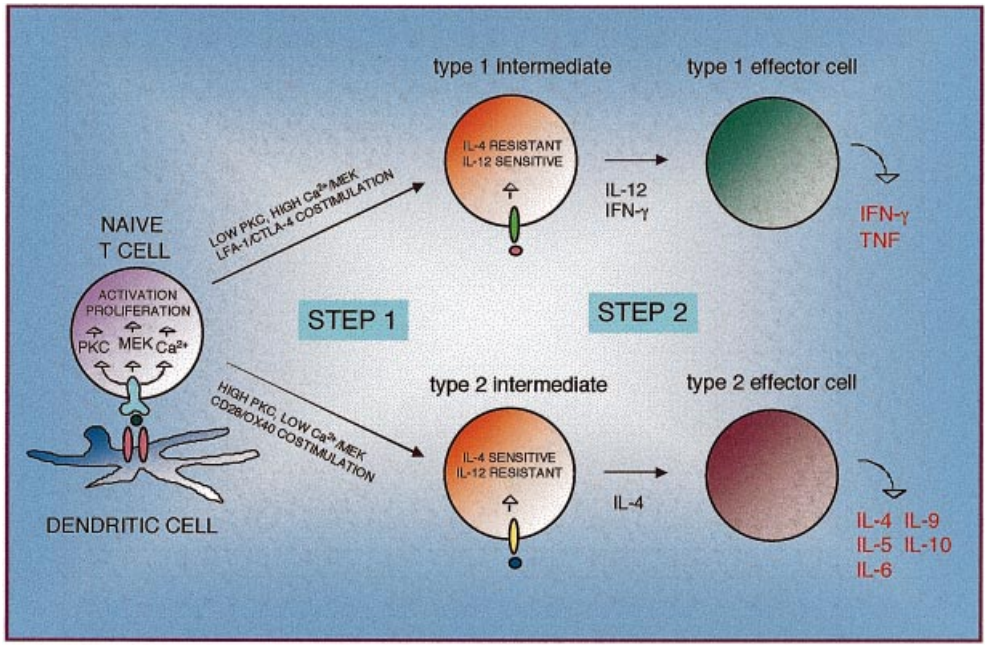

Figure 1. A two-step model of type 1/type 2 effector T-cell differentiation. Naive T cells are stimulated through immune synapses capable of transducing differential signalling patterns. The balance of T-cell receptor (TCR)-induced and costimulatory signals received activates cells into an intermediate stage in the type 1 or type 2 pathway. Sensitivity to critical cytokines interleukin (IL)-4 and IL-12 is modulated via changes in receptor expression or signals downstream of the receptors. Differentiation to mature type 1 and type 2 effectors then proceeds in response to cytokines. Note that CD8 T cells do not require IL-12 for type 1 development. CTLA-4, cytotoxic T-lymphocyte antigen-4; IFN-γ, interferon-γ; LFA-1, lymphocyte function-associated antigen-1; MAP kinase kinase (MEK); PKC, protein kinase C; TNF, tumour necrosis factor.

development.[58] Alternatively, primary stimuli could directly induce the expression of transcription factors that are selectively expressed in Th1 or Th2 cells and which induce type or type 2 differentiation independently. These include the guanosine triphosphatase Rac2, which is selectively expressed in Th1 cells and activates the IFN-γ promoter via NF-κB and p38 MAP kinase.[59] T-bet is another Th1-specific factor that potently induces IFN-γ production, even in fully differentiated Th2 or Tc2 cell lines, and is key to initiating the Th1 pathway.[60] The Th1-specific factor ERM can co-operate in IFN-γ gene induction.[61] Such Th1 transcription factors are generally induced by IL-12 signalling via STAT-4, e.g. IFN regulating factor-1 (IRF-1).[62] Another transcription factor, GATA-3, appears to be a critical control point where Th2 cell development is initiated, as its expression in STAT-6-deficient cells can fully restore Th2 development.[27,63] As well as initiating Th2 cytokine expression, it suppresses Th1 development directly without the need for IL-4.[64] GATA-3 has a repressor, repressor of GATA (ROG), which is also up-regulated by T-cell activation.[65] Additional transcription factors c-maf, JunB and nuclear factor of activated T cells c1 (NFATc1) are involved in switching on the Th2 programme.[66–68] GATA-3 induces IL-4 gene expression via c-maf, but directly targets the IL-5 promoter.[69]

The above data suggest that effector cell development is effectively a two-stage process in which the TCR-transduced stimulus can induce activated cells with a propensity for type 1 or type 2 development; these are then matured by the action of the cytokines IL-4 or IL-12 to become fully active effectors with the capacity for high-level cytokine production. During the initial stage of differentiation, lasting ≈ 2 days *in vitro*, both IL-4 and IFN-γ mRNAs are transcribed, and further development involves termination of the expression of one of the genes.[70] While IL-4 is usually required for generation of a Th2 response, in some models Th2 cells can be generated in IL-4/IL-4R or STAT-6 knockout mice.[71] This seems to confirm that IL-4 acts at a later stage of differentiation to stabilize Th2 development. Similarly, a STAT-4/IL-12-independent pathway for Th1 development has been demonstrated using knockout mice.[72] A hypothetical two-stage model for T-effector differentiation is shown in Fig. 1.

Costimulatory signals are widely reported to modulate type 1/type 2 immunity. CD28 ligation can dramatically favour the development of Th2 cells, perhaps by enhancing IL-4 production[73] or by direct activation of STAT-6.[74] An absence of CD28 signalling results in a defect of the Th2 responses,[75] and CD28 also induces Tc2-cell generation.[73] CD40–CD40L interaction selectively induces Th1 cells but this is a result of the production of IL-12 from APCs.[75] OX40–OX40L interaction favours Th2 responses[76,77] as do CD30–CD30L[78] and CD4 signals.[79] In contrast, LFA-1 signals appear to potently switch cells towards the Th1 phenotype,[80] and CTLA-4 engagement favours Th1 differentiation as well as suppressing the T-cell response.[81] The roles of costimulatory signals in type 1/type 2 development appear secondary to the TCR-transduced stimulus, as CD28 fails to induce Th2 cells in the presence of a high-affinity peptide ligand[38] and can also result from differential cytokine production. However, it is possible that signals unique to costimulatory receptors independently control differentiation. In this regard it is interesting to note that LFA-1

costimulation induced JNK activation, which might favour Th1 responses.[4,82]

In addition to their role as specialized naive T-cell activators, DCs can influence type 1 versus type 2 differentiation. DC phenotypes that elicit Th1 or Th2 development have been described as DC1 and DC2, respectively. Distinct subsets of DCs (distinguishable by their expression of CD8α) have been described in the mouse, which selectively induce Th1 or Th2 responses as a result of their differential secretion of IL-12.[83] A functional dichotomy has also been proposed for human DCs – lymphoid DCs expressing high levels of IL-3Rα and defective IL-12 secretion promote Th2 development via an IL-4-independent mechanism.[84] However, DCs that selectively prime Th1 or Th2 responses may not always originate from distinct lineages as IL-12 and IL-10 secretion can be directly regulated by T cells via signalling through the osteopontin (Eta-1) molecule on T cells, which binds an integrin receptor and CD44 to promote IL-12 and suppress IL-10 production, respectively.[85] CD40 ligation is also crucial to DC maturation and IL-12 synthesis,[86] so T-cell–DC interaction involves bidirectional dialogue mediated by both surface molecules and cytokines.

PLASTICITY AND STABILITY OF TYPE 1 AND TYPE 2 EFFECTORS

The differentiation of effector cells into the Th1 and Th2 phenotypes has been shown to involve permanent changes in chromatin structure in genetic loci of Th1 and Th2 cytokine genes. This gene remodelling process involves demethylation of DNA sequences in the IL-4 gene locus in Th2 cells.[87] Such permanent rearrangement of DNA to ensure terminally differentiated cells with stable phenotypes probably requires multiple (up to 13) cell divisions.[88] In contrast, emergence of cells secreting IL-4 or IFN-γ requires around seven divisions.[89] These observations establish type 1 and type 2 as *bona fide* differentiative pathways rather than phenotypes that are modulated by the environment of a cell. This is further confirmed by the existence of stable cell-surface markers characteristic of Th1 or Th2 cells. The ST2L molecule is permanently expressed on Th2 cells, associating closely with IL-4-secreting cells.[90] Furthermore, knockout mice indicate that this molecule is functionally important for Th2-induced eosinophil recruitment and may be important for Th2 development.[91] The signalling component of the IL-12 receptor is a marker for Th1 cells and is of obvious functional significance. The expression of stable cell-surface markers is therefore another key step in T-cell differentiation, which reinforces functional divergence of subsets. Th2 cells rapidly down-regulate expression of the IL-12Rβ2, reinforcing their polarized phenotype.[57] However, Th2 cells cannot be converted to a Th1 phenotype, even when expressing transgenic IL-12Rβ2.[92] Even fully differentiated effectors, analogous to *in vitro*-generated long-term clones, can be induced to alter their behaviour by cytokines, presumably to allow a degree of flexibility in cytokine production so that even chronic responses can be regulated if necessary. For example, IL-12 induces IFN-γ production in Tc2 or Th2 clones,[93,94] and IL-4 suppresses IFN-γ production from fully differentiated Th1 cells.[95] TCR signalling may also play a role in lineage commitment. The patterns of TCR signalling that induce selective development of type 1 or type 2 cells apparently become imprinted on the fully differentiated effector cells, as both Th2 and Tc2 clones exhibit much weaker calcium signals than their type 1 counterparts when stimulated identically by anti-CD3.[40,96,97] Th1 cells, but not Th2 cells, also signal via JNK2 MAP kinase.[98] This phenomenon may also contribute to the stability of type 1 and type 2 phenotypes as altering the antigenic peptide might fail to modulate TCR signalling in differentiated cells provided that sufficient engagement is achieved.

DIFFERENCES IN THE DEVELOPMENT OF CD4, CD8 AND γδ T-CELLS

Initial activation of CD4 and CD8 T cells differs in that different costimulatory signals appear to be critical for each subset. CD40L triggering is more effective at costimulating CD4 cells,[99] while LFA-1–ICAM interaction is a superior, and perhaps critical, signal for CD8 cells.[100,101] CD28 can costimulate both subsets, but in CD8 cells this signal can be replaced by those from IL-6 and TNF-α;[102] CD28 signalling also seems to be unable to prevent anergy in CD8 cells in the same way as it does for CD4 cells.[103] 4-1BB has also been proposed as a selective activator of CD8 cells.[104] It has been suggested that CD8 cells can develop into fully active effectors in the complete absence of costimulation if the primary stimulus is sufficient.[105] This lack of dependence on costimulation is more apparent once effector cells are developed, when CD8 cytotoxic effectors need to attack antigen-bearing non-professional APCs with the highest possible efficiency.

CD8 T cells display a clear bias towards the type 1 cytokine phenotype; so much so that Tc2-type cells were initially described more than 6 years after Th1/Th2.[106] A molecular explanation for this bias has now been put forward, in that CD8 cells have no requirement for STAT-4 signalling via IL-12 in order to develop into Tc1 effectors,[107] and IL-12 serves only to enhance IFN-γ production.[23] Regulation of the Tc1/Tc2 phenotype is also regulated by TGF-β in that IL-4 can act to promote Tc1 development and cytotoxicity in the presence of TGF-β.[108] IL-2 is also distinctly regulated in CD8 cells, while generally associating with the Th1 CD4 phenotype. In CD8 cells, IL-2 producers, generated at a much lower frequency than in the CD4 subset, are strongly suppressed by IL-4, so that IL-4 induces a state of anergy in CD8 cells.[25,109] The tight regulation of IL-2 production explains why CD8 responses are often dependent on CD4 T-cell help.[110] This help is dependent on CD4 cells activating APCs via CD40 so that they can effectively stimulate CD8 cells,[111] as well as providing cytokines.[112] In some cases, however, CD8 responses do not require such help.[113] The differences that can emerge between CD4 and CD8 cytokine profiles allow bidirectional regulation between the two subsets. Th2 cell-derived IL-4 probably assists the development of Tc2 cells in allergic states.[114] Conversely, CD8 T-cell responses often regulate concomitant CD4 responses, suppressing Th2 immunity and enhancing Th1 responses. This is associated with the relative propensity of CD8 cells to secrete high levels of IFN-γ.[115,116]

γδ T cells mediating immunity to unconventional antigens are capable of differentiating into subsets comparable to αβ Th1 and Th2 cells in both cytokine profile and function.[117] Like CD8 cells, they are biased towards the type 1 pathway compared with their CD4 αβ counterparts. Unlike CD8 cells,

however, this bias is the result of persistent expression and function of the IL-12 receptor, which ensures continued IFN-γ production in the presence of IL-4.[118]

TYPE 1/TYPE 2 AS A POPULATION PHENOMENON

The integrity of the Th1/Th2 paradigm has been challenged by observations that acquisition of expression of any particular cytokine during CD4 T-cell development is stochastic.[119] This means that the probability of a T cell expressing IL-4 and IFN-γ simultaneously is no less than predicted by random association. CD8 T-cell cloning also results in clones expressing virtually all possible combinations of cytokines,[93] and intracellular cytokine analysis reveals heterogeneity of cytokine profiles in newly generated effector cells.[120] The emergence of Th1 or Th2 phenotypes therefore depends on increased probabilities of each Th1 or Th2 cytokine being expressed in the population as a whole, rather than co-ordinate regulation of cytokines in individual cells. This process occurs gradually during an immune response *in vivo*, despite rapid Th1/Th2 polarization being achievable *in vitro*. This presumably allows more sophisticated regulation of an emerging immune response with a greater variety of cytokines being available. Many cells may remain uncommitted to either type 1 or type 2 pathways except after chronic exposure to antigen *in vivo*. CD4 T cells cultured in non-polarizing conditions *in vitro* display a mixed Th1/Th2 profile, as demonstrated by intracellular cytokine staining.[40] Interestingly, isolation of viable cytokine-positive cells suggests that once an individual cell starts to secrete IL-4, it becomes committed to the type 2 pathway.[27] Cells simultaneously producing IL-4 and IFN-γ are rare in most systems and the Th0 phenotype is therefore largely a population phenomenon.[120] It has also been suggested that Th1 and Th2 cells *in vivo* may arise from different precursors, which would mean that regulation of individual cell fates was less important in immune regulation.[121]

T-CELL SUBSETS OTHER THAN TYPE 1 AND TYPE 2

A number of cytokine profiles have been described that are distinct from the classical type 1 and type 2 phenotypes. High-level secretion of TGF-β, along with varying quantities of IL-4 and IL-10, has been termed the Th3 phenotype and correlates with a suppressor phenotype that is induced during oral tolerance.[122] In addition to such peripheral differentiation, regulatory cells expressing a particular cell surface phenotype ($CD25^+$, $CD4^+$, CTLA-4^+) appear to emerge from the thymus as a discrete subset and act to suppress autoimmune responses via production of TGF-β, IL-4 or IL-10.[123] However, these cells require exposure to antigen in the periphery before they differentiate into active suppressor cells.[124,125] A similar regulatory cell develops in response to gut antigens and prevents intestinal inflammation.[126,127] It appears that both CD4 and CD8 regulatory subsets producing similar cytokines can contribute to oral tolerance.[128] Differentiation into Th3-like cells may require IL-4.[129] Another suppressor cell subset, known as T regulatory-1 (Tr1), is characterized by high-level IL-10 and IL-5 secretion and is a slow-growing cell type induced by culture in the presence of IL-10.[130] Differentiation of CD4 T cells into regulatory phenotypes may be induced by the interaction of two receptors – Serrate-1 (expressed by APC) and Notch-1 (expressed on T cells) – that also determine cell fates during thymic T-cell differentiation.[131] It is not entirely clear whether regulatory cells are a result of differentiation into separate lineages or caused by differential signalling. However, it is evident that IL-10 production can be regulated independently from classical type 1 and type 2 cytokines in both CD4 and CD8 subsets, can be induced by both IL-4 and IL-12, and that this is an important aspect of immune regulation.[132,133] It should be noted that regulatory cells may not selectively secrete suppressive cytokines but mediate their effects via induction of apoptosis in reactive cells via CD95 ligation.[134] Such cells have recently been described in allograft rejection and display a novel $CD4^-$ $CD8^-$ phenotype.[135]

GENERATION OF MEMORY CELLS

Another potential result of naive T-cell activation is the generation of memory cells. These differ from effector cells in that they are smaller quiescent cells that require priming and restimulation before exerting effector function. Their ability to mediate immunological memory is a result of their increased clone size and their ability to proliferate and differentiate very rapidly. However, they have a lower threshold of activation than naive cells and express different adhesion molecules and chemokine receptors that affect their recirculation and homing properties so that they are ready to respond to further presence of antigen in tissues. However, recent evidence clearly indicates that memory cells are derived directly from effector cells rather than as a separate lineage from naive T cells.[136,137] Some memory cells express lymph node homing receptors that allow them to populate lymph nodes for extended periods of time. These 'central memory' cells can differentiate rapidly into 'effector memory' cells after re-encountering antigen, and then express receptors for migration into inflamed tissues.[138] Effector cells therefore choose between death and the memory pathway once formed; the signals that control this choice are not entirely clear but ligation of OX40 along with other APC-derived factors can greatly amplify the memory pool.[139] Memory cells also express increased levels of the survival factor bcl-2.[140] IL-15 can selectively promote survival of CD8 memory cells,[141] and death of activated T cells can be prevented by all the cytokines that signal through the IL-2R common γ-chain (IL-2, -4, -7 and -15) and this could contribute to maintaining a memory pool.[16] Memory cells are able to recall the pattern of cytokine production they previously acquired in addition to their antigen specificity.[137]

UNRESPONSIVE PHENOTYPES AND ACTIVATION-INDUCED CELL DEATH

Induction of anergic or unresponsive T cells is classically attributed to TCR-mediated stimulation in the absence of costimulatory signals, leading to a block in IL-2 secretion.[142] Lack of CD28 or 4-1BB[143] signals can result in anergy, but IL-10 has more recently been demonstrated to interfere with T-cell activation and induce an anergic T-cell phenotype.[144] CD28 signalling prevents anergy by up-regulating IL-2 secretion.[142] In addition, activation of T cells leads to up-regulation of CTLA-4 and, if this is ligated, an anergic phenotype can develop.[145] Anergy can be induced by altered

peptides by inducing reduced levels of TCRζ phosphorylation, transducing a distinct signal through the TCR which blocks IL-2 production,[146] and anergic cells retain defective TCR signalling which results in faulty induction of the IL-2 gene.[147] In some models anergy cannot be reversed by addition of IL-2 or other γ-chain cytokines and this has been associated with defective signalling via Jak-3 and STAT-5 through the common γ-chain itself.[148] In CD8 cells, lack of CD4 help in the form of IL-2 or other signals may lead to CD8-cell anergy.[110] It has been speculated that a lack of secondary stimulation after initial T-cell differentiation, for example by B cells, could prevent full effector function, leading to anergy or death because CTLA-4 is not down-regulated.[18] The link between CTLA-4 signalling and TGF-β may be critical here. TGF-β seems to be crucial for the maintenance of an unresponsive state towards self-antigens as abrogation of TGF-β receptor signalling results in autoimmunity.[149] Paradoxically, TGF-β in combination with IL-2 prevents apoptosis of Th2-effector cells, allowing rapid expansion in effector-cell numbers.[150] DCs undoubtedly play a role in maintaining T-cell tolerance by processing tissue self-antigens, probably from apoptotic cells,[151,152] and in the absence of inflammatory/danger signals,[153] presenting them to both CD4 and CD8 cells in a tolerogenic fashion.[154,155] This involves transfer of antigen from migratory to resident lymph node DCs.[156]

Another important aspect of T-cell differentiation is the development of altered susceptibility to regulation by induction of apoptosis. Activation of T cells and their subsequent exposure to IL-2 programmes them for activation-induced cell death (AICD).[157,158] This process ensures that on secondary stimulation of effectors, a proportion of the cells undergo apoptosis, limiting the expansion of cells and providing an important mechanism of tolerance.[159] This phenomenon most commonly involves CD95–CD95L interaction and occurs much more readily in Th1 cells, Th2 cells being resistant, perhaps owing to differential TCR signalling or enhanced expression of the protective Fas-associated protein-1 (FAP-1) molecule.[160] Costimulatory signals may prevent AICD, and up-regulation of additional costimulators (such as 4-1BB) after activation could help prevent premature apoptosis.[161] AICD is distinguished from another form of T-cell apoptosis that occurs when T cells are starved of the cytokines and other signals they need for continued turnover or survival. Unlike AICD, this type of death can be prevented by IL-2 or other cytokines that signal via the common γ-chain. IL-15 can prevent apoptosis but appears to direct the cells into an anergic phenotype,[162] while IFN-β reverts activated cells to a resting state with prolonged survival.[163] IFN-γ can enhance AICD by inducing nitric oxide production from APCs.[164] Eventually, in old age, the number of T cells with defective signalling[165] and reduced proliferative capacity[166] increases. These cells often exhibit an anergic phenotype.[167] Thus, the final stage of differentiation in the long life of some T cells may be the development of senescence associated with chromosomal telomere shortening.[168] This phenomenon might result in defective immune responses in extreme old age.

CONCLUSIONS

Multiple steps in differentiation occur after engagement of the TCR complex of peripheral T cells by an antigenic peptide–MHC complex. Complex information can be transmitted into the T-cell cytoplasm, influencing the critical choice between effector response or tolerance, and modulating subsequent responses to key immunoregulatory cytokines. Divergent differentiation, enforced by genetic reprogramming, ensures that an appropriate class of immunity is generated, while differential regulation of apoptosis controls the expansion of effector cells and helps to develop an appropriately sized pool of memory cells ready for secondary responses. Manipulation of the multiple intracellular signalling pathways involved in these regulatory processes may allow much more effective control of immunopathologies than has previously been possible.

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