Interleukin-7: master regulator of peripheral T-cell homeostasis?

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Recent evidence has implicated interleukin-7 (IL-7) as a master regulator of T-cell homeostasis, based upon its essential role in the homeostatic expansion of naive T-cell populations in response to low-affinity antigens (Ags) and its capacity to enhance dramatically the expansion of peripheral T-cell populations in response to high-affinity Ags. Furthermore, T-cell-depleted humans have a unique inverse relationship between the peripheral CD4+ T-cell count and the level of circulating IL-7. Together, these data suggest that increased amounts of IL-7 become available following T-cell depletion, thus, enhancing the high- and low-affinity Ag-driven expansion of the population of residual, mature T cells and boosting thymic regenerative capacity, as a means to restore T-cell homeostasis.

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Primary selection of the T-cell repertoire occurs within the thymus, but significant fine-tuning of the repertoire can also occur in the periphery, as a result of preferential expansion or contraction of T-cell populations in response to cognate antigen (Ag), self Ag or other homeostatic signals. Recent work by several laboratories has identified a crucial role for engagement of the MHC in the survival and maintenance of peripheral, naive T-cell populations[1], and cytokine support has been shown to be important for the maintenance of memory T-cell populations[1,2]. In the setting of T-cell depletion, these peripheral mechanisms of T-cell homeostatic regulation are exaggerated and play a central role in restoring T-cell homeostasis.

Immunologists have long appreciated that T-cell-depleted (TCD) hosts engraft and expand adoptively transferred T-cell populations efficiently[3–5], but the mechanisms responsible for this phenomenon have remained largely unknown. Recent studies have shown that interleukin-7 (IL-7), in addition to its well-known effects on the early development of lymphocytes, also functions as a crucial regulator of peripheral T-cell homeostasis by modulating the expansion of peripheral T-cell populations in states of T-cell depletion[6–8]. In the model that is emerging, T-cell depletion results in increased levels of stromally produced IL-7 (Refs 9–11), which serve to increase the proliferation of peripheral T cells in response to both high-affinity and low-affinity Ags, thus, increasing peripheral homeostatic expansion (Fig. 1)[6,7]. In this article, we will begin with an overview of IL-7, followed by a synopsis of recent advances in our understanding of the biology of peripheral homeostatic T-cell expansion and the central role that IL-7 plays in modulating this process. We will then review recent human studies showing that levels of IL-7 are increased in response to peripheral T-cell depletion, further implicating IL-7 as a master regulator of peripheral T-cell homeostasis.

IL-7: background and biology

IL-7 has been shown unequivocally to be a requisite cytokine for lymphopoiesis, because animals deficient in IL-7 are essentially devoid of B- and T-cells[12–15]. Clinical studies of patients with congenital immunodeficiencies have confirmed a requirement for IL-7 for primary T-cell development in humans; however, IL-7 is not required for the development of B cells in humans[16]. Because of the profound effects of IL-7 on developing lymphocytes, it is not surprising that these effects have been the primary focus for studies of this cytokine[17–19]. Furthermore, the inability to study peripheral T-cell biology directly in IL-7-deficient mice (owing to a profound deficiency of peripheral T cells) slowed the investigation of the potential role that this cytokine might play in peripheral T-cell homeostasis.

The IL-7 receptor (IL-7R) is comprised of an α chain (IL-7Rα), shared with thymic stromal lymphopoietin (TSLP), and a common γ chain, which is shared by the receptors for IL-2, IL-4, IL-7, IL-9

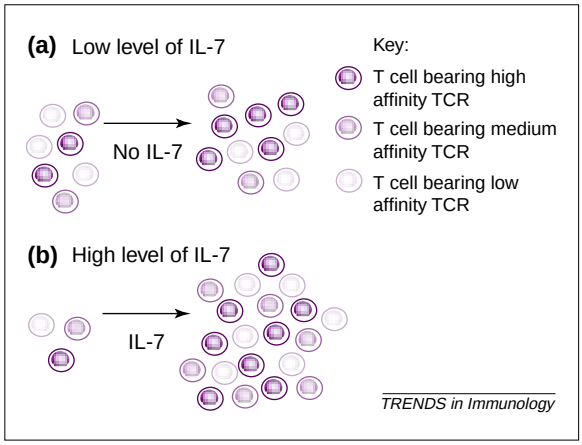

Fig. 1. The relative affinity of the interaction between peptide–MHC and T-cell receptor (TCR) required for T-cell proliferation appears to vary depending upon the relative availability of interleukin-7 (IL-7). Two extremes of this continuous spectrum of TCR affinity and availability of IL-7 are illustrated. (a) In settings where IL-7 is not available, only high-affinity 'cognate' recognition induces T-cell proliferation. (b) However, in states of IL-7 excess, such as occurs in T-cell-depleted hosts, T cells bearing TCR specificities that recognize peptide–MHC with low affinity can be induced to proliferate. The net result is a relative increase in the frequency of antigen-specific T cells capable of responding to a given antigen, which might compensate partially for the depletion of T cells.

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and IL-15 (Ref. 20). IL-7R is present on developing thymocytes and almost all mature T cells. Although the expression of IL-7R is diminished transiently following the activation of T cells by cognate Ag (Ref. 6), both $CD4^+$ and $CD8^+$ naive and memory T cells express IL-7R (Ref. 7). *In vitro*, IL-7 functions as a costimulator for the activation of mature T cells[21]. When a potent T-cell receptor (TCR) stimulus is supplied with adequate costimulation, the effects of IL-7 are relatively mild; however, following suboptimal signaling through the TCR, such as with suboptimal concentrations of anti-CD3 antibody (Ab), application of exogenous IL-7 increases the T-cell yield substantially compared with control cultures[22].

The mechanisms by which IL-7 exerts its effects on peripheral T cells appear to be multifactorial. First, IL-7 potently inhibits programmed cell death by the up-regulation of expression of members of the bcl-2 family of anti-apoptotic molecules[23,24] and also potentially, the transcription factor lung Kruppel-like factor (LKLF), which functions as a T-cell survival factor[25]. This trophic effect of IL-7 is an important component of its action on developing thymocytes[18,19] and also, improves the survival of T cells in long-term cultures[26]. Furthermore, when IL-7 is absent during primary T-cell activation *in vivo*, the generation of memory cells is greatly reduced[6], potentially owing to impaired survival of responding T-cell clones. Thus, the inhibition of programmed cell death appears to be a primary effect of IL-7, not only on developing lymphocytes but also, on peripheral T-cell populations. Second, IL-7 increases the cycling of mature T cells following suboptimal stimulation with anti-CD3 Abs *in vitro*[21,22], potentially, by up-regulating the expression of the gene encoding IL-2 (Ref. 27). Third, IL-7 enhances the cytolytic function of mature T cells[28]. Finally, recent evidence has shown that some component of the effect of IL-7 on peripheral T-cell responses involves IL-7-mediated signaling by Ag-presenting cells (APCs), which appears to enhance T-cell–APC interactions through as yet uncharacterized mechanisms[22]. Thus, IL-7 acts through a variety of mechanisms to increase the proliferative response and survival of mature T cells following encounter with Ag. These effects are most pronounced following suboptimal signaling through the TCR, such as might occur during an encounter with low-affinity foreign- or self-peptides.

Peripheral homeostatic expansion in TCD hosts

The adoptive transfer of mature T cells into T-cell-replete mice does not induce a sufficiently chimeric state to study the biology of the transferred cells. To facilitate the 'take' of the adoptively transferred T cells, it is common practice to use TCD hosts, in essence, to provide 'space' for the transferred T cells[3–5]. The expansion of mature T-cell populations in TCD hosts has been variably termed 'peripheral expansion' or 'peripheral homeostatic expansion' (PHE). This expansion is primarily responsible for the restoration of T-cell homeostasis following T-cell depletion in athymic mice[29] and appears to be largely responsible for the early phases of the restoration of T-cell numbers following T-cell depletion in adult humans[30–32].

The role of interactions with MHC and Ag in driving the process of PHE in TCD hosts has been investigated recently by several groups. Initial studies emphasized the role of cognate Ag in driving this process by showing that in the absence of cognate Ag for specific transgenic TCRs, peripheral expansion of $CD8^+$ and $CD4^+$ T cells did not occur[33,34]. These studies also pointed out that in the setting of T-cell depletion, responses to cognate Ag are greatly exaggerated compared with those observed in T-cell-replete hosts[34]. Thus, when challenged with the same Ag and the same number of responding T-cell populations, TCD hosts generate substantially greater numbers of Ag-specific T cells than T-cell-replete hosts.

However, results from studies of cognate Ag do not explain what drives PHE in TCD hosts given syngeneic inocula in the absence of cognate Ag. Because interactions with MHC are required for the survival of naive $CD4^+$ and $CD8^+$ T cells in both TCD and T-cell-replete hosts[1], it was predicted that cross-reactive environmental Ags present in syngeneic hosts drive the PHE of naive cells. A series of recent studies using elegant models that control the availability of peptide to transferred TCR-transgenic cells has allowed direct testing of this prediction by studying the role of high-affinity versus low-affinity Ag in driving the expansion of T-cell populations in the presence or absence of T-cell depletion.

In the first model, the expansion of ovalbumin-specific-TCR-transgenic T cells, following exclusive exposure to high-affinity cognate peptide in TCD hosts, was compared with exposure to a low-affinity peptide in TCD and T-cell-replete hosts. Results showed that TCD hosts have a unique ability to respond to the low-affinity peptide compared with T-cell-replete hosts[35]. Although the degree of expansion induced by low-affinity peptide was less than that induced by high-affinity peptide, these results illustrate that proliferation in response to low-affinity Ags helps to maintain the diversity of the TCR repertoire in the setting of T-cell depletion.

Simultaneously, another report showed that $CD4^+$ T cells derived from H2-M^- hosts [which positively select $CD4^+$ T cells in the thymus based upon their specificity for a single species of peptides derived from class-II-associated invariant chain peptide (CLIP)] underwent PHE only in animals with peripheral expression of the selecting peptides, but not in normal B6 mice. By contrast, $CD4^+$ T cells from H2-M^+ mice (which positively select $CD4^+$ T cells in response to a wide array of peptide specificities) did not undergo homeostatic expansion in H2-M^- hosts (which exclusively present CLIP-derived peptides in the

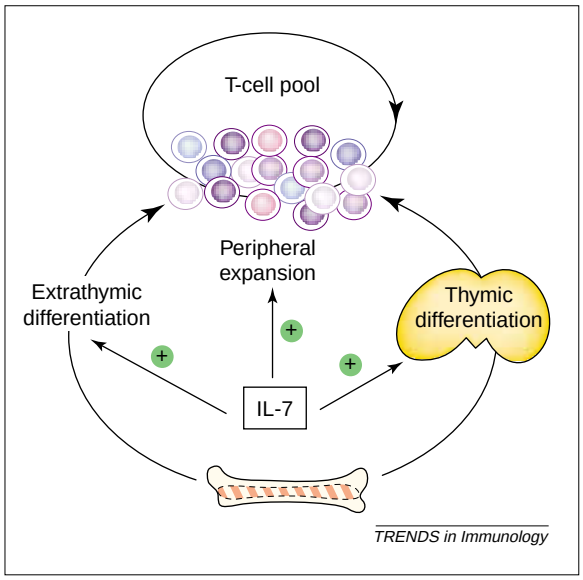

Fig. 2. Following T-cell depletion, interleukin-7 (IL-7) enhances thymic-dependent T-cell regeneration and the peripheral expansion of mature T cells. In addition, emerging evidence suggests that IL-7 might also induce extrathymic T-cell development[65].

periphery)[36]. This work led to the novel suggestion that positive thymic selection might play an important role in generating a TCR repertoire capable of optimal peripheral survival, by selecting cells with the capacity to survive by their response to self peptides presented in the periphery. Taken together, these studies illustrate that the enhanced proliferative responses of T cells to cognate or high-affinity Ags and the recruitment of T cells with receptors for low-affinity Ags into the proliferative pool contribute to the unique immunobiology of TCD hosts.

In these experimental systems, the biological distinction between high-affinity (e.g. cognate) and low-affinity (e.g. self) peptides is significant and measurable. For example, there was an approximate sevenfold difference in the affinity of the high- versus low-affinity peptides for the ovalbumin-specific TCR used in the studies described[35]. In general, although both high- and low-affinity interactions give rise to cells bearing a 'memory' phenotype (i.e. $CD44^{hi}CD122^{+}Ly6C^{+}$), the proliferative response to low-affinity peptides in TCD hosts occurs at a diminished rate when compared with high-affinity peptides, and low-affinity peptides do not induce the expression of activation markers (e.g. CD69, CD25 and CD71)[37–39]. These T cells from TCD hosts are capable of generating fully fledged responses if challenged subsequently with cognate Ag (Refs 37–39). Interestingly, upon recovery of normal numbers of peripheral T cells, $CD8^{+}$ T cells specific for self peptides appear to revert to a naive phenotype, whereas in chronically TCD hosts, the memory phenotype persists[37]. With regard to the acquisition of effector function, proliferation in response to self Ag has been variably described to occur either without the induction of effector function[38,39] or with only transient effector function[37], whereas cognate Ag induces robust effector function[38,39].

IL-7 is a crucial cytokine during peripheral homeostatic expansion

The administration of IL-7 increases the rate of T-cell immune reconstitution following bone-marrow transplantation and/or cytotoxic chemotherapy in mice[40–42]. Because IL-7 has potent effects on developing thymocytes as well as peripheral T cells, experiments were undertaken to distinguish the ability of IL-7 to modulate thymopoiesis from its effects on the expansion of peripheral T-cell populations. IL-7 increased substantially both thymic-dependent T-cell regeneration and the peripheral expansion of mature T-cell populations following T-cell depletion[8] (Fig. 2). Although IL-7-mediated signaling through the common γ chain is not required for Ag-induced proliferation[43], the Ag-driven expansion of high-affinity clones was increased substantially in TCD mice treated with IL-7. Furthermore, in the absence of cognate Ag, IL-7 was the only cytokine tested that was capable of increasing the rate and/or extent of PHE in TCD hosts, whereas IL-2, IL-3, IL-6 and IL-12 had no effect[8]. In a separate report, PHE within lymphoid organs *in vitro* could be enhanced by IL-4, IL-7 or IL-15 (Ref. 7), and in a second *in vivo* study, IL-12 was shown to increase the rate and/or extent of PHE (Ref. 44). Thus, in the setting of T-cell depletion, the provision of exogenous IL-7 is noted consistently to enhance PHE, and some reports have also shown that IL-4, IL-12 and IL-15 are active in this regard.

In studies of T-cell-replete hosts, the administration of IL-7 following the transfer of syngeneic T-cell populations increased the 'take' of these cells, essentially, by 'fooling the host into thinking that it is TCD' (Ref. 8). IL-2 has a modest capacity to increase PHE in T-cell-replete hosts, but IL-7 is much more potent in this regard. Thus, an increased availability of IL-7 is sufficient to induce PHE even in the absence of T-cell depletion. Also, IL-7 therapy improved substantially immune competence in TCD hosts, rendering them able to reject skin grafts mismatched for minor histocompatibility Ags (Ref. 22). Taken together, these results identify IL-7 as a potent modulator of T-cell immune reconstitution, able to restore immune competence through its combined effects of increased thymic output and enhanced PHE of T cells.

Further insight is derived from studies in which IL-7 is unavailable during PHE. When $IL\text{-}7R^{-/-}$ T cells are transferred into wild-type TCD hosts[6], or wild-type T cells are transferred into $IL\text{-}7^{-/-}$ TCD hosts[7], the proliferative response of T cells to low-affinity Ags is absent. Thus, IL-7 is required for the proliferation of naive T cells with TCRs specific for low-affinity Ags

Table 1. Known roles of selected cytokines in modulating peripheral T-cell homeostasis[a]

Cytokine	Thymic T-cell production				Expansion of T cells in response to cognate Ag				Expansion of naive T cells in response to low-affinity Ag				Expansion of memory cells			
	Required	Refs	Augments	Refs	Required	Refs	Augments	Refs	Required	Refs	Augments	Refs	Required	Refs	Augments	Refs
IL-2	–	20	+	8	–	43	+	20	–	7	–	7,8	–	2	–	2
IL-4	–	20	ND		–	43	+	20	–	7	+	7	ND		ND	
IL-7	+	12,13	++	8	–	43	+	8,20	+	6,7	++	7,8	–	2,6	+	6
IL-12	–		–	8	ND		+		ND		+/–	8,44	ND		ND	
IL-15	–	20	ND		–	43	+	4	–	7	+	7	+	2	+	2

[a]Abbreviations: Ag, antigen; IL, interleukin; ND, not determined.

that occurs in TCD hosts. Similar results were observed by Boursalian *et al.*, who showed that the combined inhibition of IL-7 and IL-4 prevented the survival of naive cells *in vitro*[45], and Tan *et al.*, who showed diminished survival of naive cells in the absence of IL-7 *in vivo*[7]. Importantly, the role of cytokines in modulating the survival of memory cells appears to be distinct from their role in regulating the survival of naive cells, because the maintenance of $CD8^+$ memory cells requires IL-15 and occurs in the absence of MHC engagement[2,46]. Interestingly, in these studies, the depletion of IL-7 also reduced the survival of memory cells *in vivo*, albeit less consistently and less potently than observed with the inhibition of IL-15. In summary, data produced to date show that IL-7 is absolutely required for T-cell proliferation in response to low-affinity ligands during PHE, and a supraphysiological level of IL-7 is sufficient to induce PHE in T-cell-replete hosts and can enhance the magnitude of expansion of naive-cell populations in response to cognate Ag. With regard to memory cells, IL-7 is a cofactor for the development of memory cells from naive precursors, and, although less potent than IL-15, IL-7 might also play some role in the maintenance of $CD8^+$ memory-cell populations. Although a complete discussion of the cytokines involved in T-cell homeostasis is beyond the scope of this review, Table 1 summarizes the known roles for selected cytokines.

Elevations in the level of IL-7 in clinical settings associated with T-cell depletion

The identification of IL-7 as a requisite factor for homeostatic expansion in response to low-affinity Ags provided clear evidence that at least a basal level of constitutive IL-7 is required for PHE to occur. However, the observations that exogenous IL-7 could potently enhance PHE in TCD hosts and induce PHE in T-cell-replete hosts raised the possibility that changes in endogenous levels of IL-7 might be responsible for modulating the differential rates of PHE observed in TCD versus T-cell-replete hosts. Furthermore, the fact that IL-7 also increases potently thymic-dependent pathways of T-cell regeneration emphasizes the potential beneficial effects of increased endogenous levels of IL-7 in TCD hosts.

Studies of circulating IL-7 in TCD populations were started by Bolotin *et al.*, who found substantial elevations in the level of circulating IL-7 in children following allogeneic bone-marrow transplantation; levels were highest in patients with the most profound T-cell depletion[9]. Follow-up studies have now been conducted in multiple clinical cohorts with T-cell depletion. A profound inverse relationship is observed between the level of circulating IL-7 and peripheral $CD4^+$ T-cell numbers, in both children and adults with HIV infection[10,11] (Fig. 3). Following effective antiviral therapy, recovery of the number of $CD4^+$ T cells is accompanied by a decline in the level of circulating IL-7. Although statistical correlations also exist between the level of IL-7 and the size of other lymphocyte subsets (e.g. $CD8^+$ T cells and B cells), the strongest correlation exists between circulating levels of IL-7 and $CD4^+$ T-cell counts. Because these patients were not depleted of $CD8^+$ T cells, these studies cannot rule out the possibility that isolated depletion of $CD8^+$ cells might also be sufficient to raise the circulating levels of IL-7. Similar relationships were not observed between counts of circulating lymphocytes and levels of IL-2, IL-4, IL-6, IL-12 or IL-15, suggesting that the relationship with IL-7 is unique[10].

In adults with normal numbers or only minimal depletion of $CD4^+$ T cells, no relationship between the level of circulating IL-7 and the number of $CD4^+$ cells is observed. However, in one cohort of patients with moderate total $CD4^+$ T-cell depletion, but more substantial depletion of the naive subset of $CD4^+$ cells, a strong inverse correlation between the level of circulating IL-7 and the number of $CD62L^+CD45RA^+CD4^+$ cells was observed, which gradually disappeared upon recovery of the naive subset[10]. Thus, the isolated depletion of either total $CD4^+$ T cells or the $CD4^+$ naive subset appears to be capable of driving elevations in the level of IL-7.

Inverse relationships were also seen in children and young adults treated with cytotoxic chemotherapy for cancer. Here, the level of circulating IL-7 increased following $CD4^+$ T-cell depletion induced by chemotherapy, and IL-7 levels returned to baseline following the recovery of $CD4^+$ T-cell numbers after the completion of therapy[10]. Similarly, in a cohort of patients with idiopathic $CD4^+$ T-cell lymphopenia, which comprises a heterogeneous group of patients with depletion of uncertain etiology[47], significant relationships between $CD4^+$ T-cell counts and circulating levels of IL-7 were

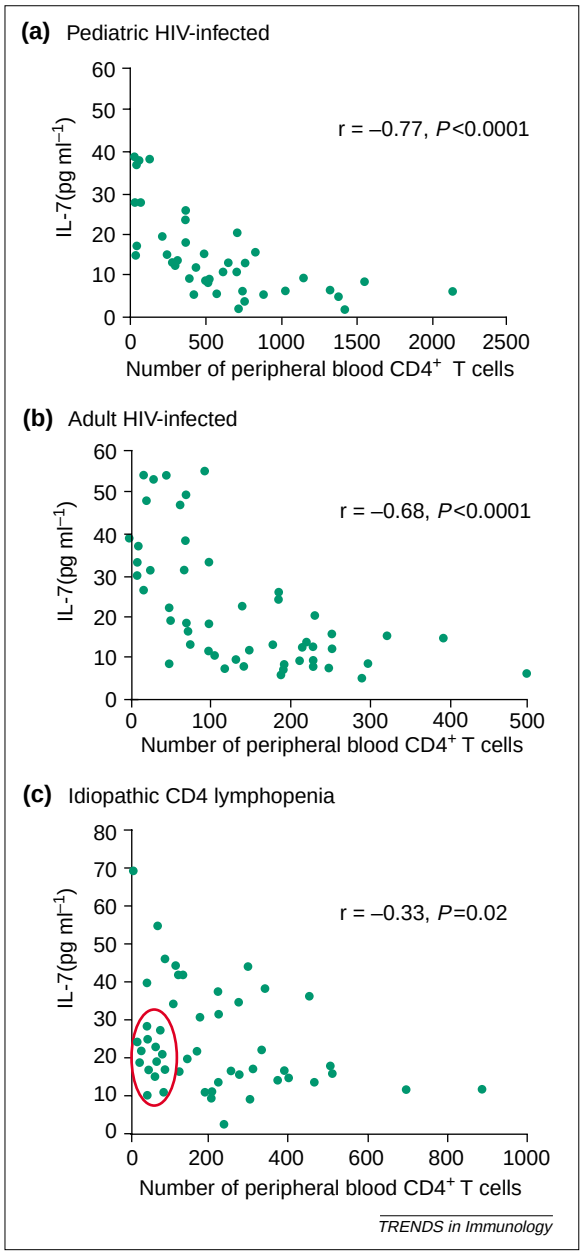

Fig. 3. Inverse relationships are observed between the levels of circulating interleukin-7 (IL-7) and peripheral-blood CD4+ T-cell counts in (a) children and (b) adults infected with HIV. (c) Similar relationships are observed in idiopathic CD4+ T-cell lymphopenia, although they are less significant ($P = 0.02$ compared with <0.0001 for children and adults infected with HIV). Circled is a population of patients with inappropriately low levels of IL-7, relative to the degree of CD4+ T-cell depletion. This finding raises the possibility that diminished availability of IL-7 might contribute to the development of CD4+ T-cell lymphopenia for some patients contained in this very heterogenous clinical group. Abbreviations: *P*, probability; r, correlation coefficient. Adapted, with permission, from Ref. 10.

observed – although the statistical significance was substantially lower than that observed during infection with HIV or following chemotherapy (Fig. 3). In this cohort, a subset of patients was observed to have relatively low levels of circulating IL-7 for the degree of CD4+ T-cell depletion, suggesting that low levels of IL-7 might have contributed to the development of CD4+ T-cell lymphopenia[10]. Recently, a subset of HIV-infected patients with unexpectedly low levels of IL-7 for the degree of CD4+ T-cell depletion has also been identified. In cross-sectional analyses, the CD4+ TCD patients with low levels of IL-7 had a diminished capacity to restore peripheral CD4+ T-cell numbers following effective antiviral therapy[48]. Further studies are necessary to confirm whether low levels of IL-7 in the face of CD4+ T-cell depletion correlate with a diminished capacity for immune reconstitution and to identify the cause of

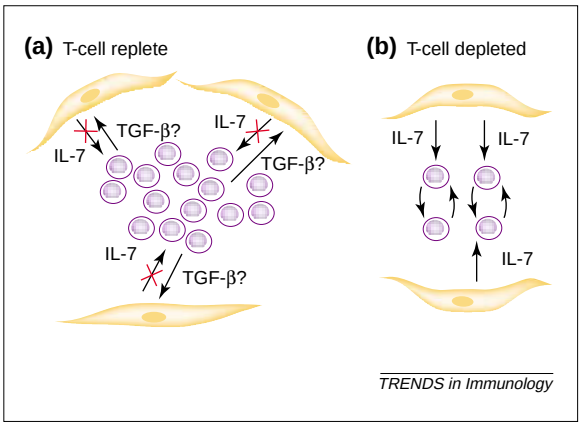

Fig. 4. Studies in clinical populations have shown that elevated circulating levels of interleukin-7 (IL-7) in settings of T-cell depletion return to base-line upon the recovery of T-cell populations. This figure suggests (a) a possible feedback loop, whereby constitutively produced soluble factors synthesized by T cells suppress the production of IL-7 by stromal cells. (b) Upon depletion of T cells within the lymphoid niche, reductions in the level of the IL-7-suppressive factor(s) would lead to increased production of IL-7. Because transforming growth factor β (TGF-β) is known to be produced by T cells and to regulate the production of IL-7 by marrow stroma[49], it is a candidate IL-7-suppressive factor.

low levels of IL-7 in some patients with depletion of CD4+ cells.

Much work remains to be done regarding the mechanisms responsible for the increased level of circulating IL-7 in TCD hosts. Currently, it is not known whether levels of IL-7 increase owing to the depletion of cells expressing IL-7R and/or reflect an increase in the production of IL-7. Circumstantial evidence suggests that increased production might be involved because: (1) there appears to be a time delay between T-cell depletion and increases in the level of circulating IL-7 (Ref. 10); (2) the expansion of the CD8+ T-cell subset that expresses IL-7R is not sufficient to lower IL-7 levels; and (3) immunohistochemical analysis demonstrates an increased level of cell-associated IL-7 in the lymph nodes of TCD hosts[11].

If T-cell depletion induces increased production of IL-7, the IL-7-producing cell and the mechanisms by which stromal cells might sense T-cell numbers remain unknown. IL-7 is produced by a wide variety of stromal and epithelial tissues. In murine studies, the IL-7-producing cell responsible for inducing the proliferation of naive cells in response to low-affinity ligands was not derived from the bone marrow[6]. Although IL-7 is produced in large quantities by thymic epithelium, the fact that similar elevations in the level of IL-7 are observed in children and adults, despite a relatively diminished thymic tissue mass in adults compared with children (Fig. 3), suggests that thymic production might not be the primary source for the circulating IL-7 observed. With regard to sensors for T-cell depletion, one possibility involves transforming growth factor β, which is known to be produced constitutively by T cells and can regulate the production of IL-7 by marrow stromal cells[49] (Fig. 4).

Potential implications of elevated IL-7 levels in TCD hosts

CD8⁺ expansions following CD4⁺ depletion: insights into 'blind homeostasis'?

Clinical and murine studies have shown that the isolated depletion of CD4⁺ T cells leads to the expansion of CD8⁺ T-cell populations[50–52]. This observation has led to the 'blind homeostasis' model of T-cell regulation, which holds that the immune system will respond to the depletion of T-cell subsets by expanding whatever population remains available. Invoking IL-7 as a master regulator of T-cell homeostasis could account for these observations, because isolated subset depletion, at least for CD4⁺ T cells, is sufficient to elevate the level of IL-7 (Ref. 10). IL-7 exerts its actions on both CD4⁺ and CD8⁺ T cells, and mice treated with IL-7 in the absence of T-cell depletion typically develop an inversion of the CD4⁺:CD8⁺ ratio, with a selective expansion of the CD8⁺ T-cell compartment[53]. Furthermore, recent studies of thymic development have shown that IL-7 selectively induces commitment to the CD8⁺ lineage[54]. Thus, the clinical observation that expansion of CD8⁺ T-cell populations typically accompanies CD4⁺ T-cell depletion is consistent with the process of immune reconstitution being driven largely by IL-7 (Fig. 5).

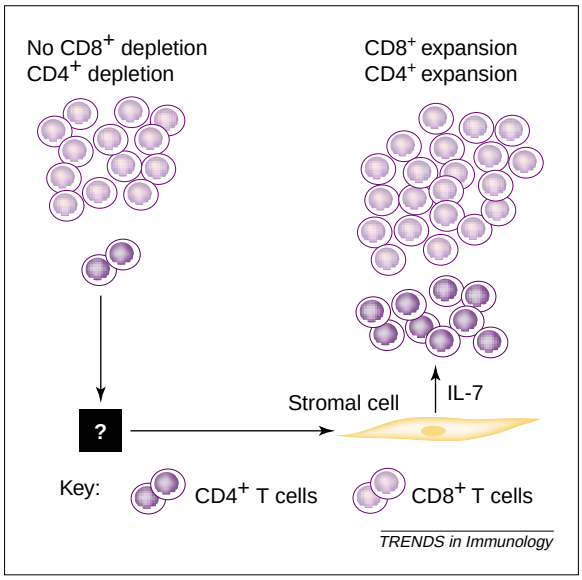

Fig. 5. Potential interleukin-7 (IL-7)-based model to explain 'blind T-cell homeostasis'. The isolated depletion of T-cell subsets (at least for CD4⁺ cells) appears to be sufficient to increase the level of circulating IL-7, presumably, by communication with stromal cells through as yet uncharacterized mechanisms. The net result of an increased level of circulating IL-7 in response to isolated CD4⁺ T-cell depletion is expansion of the remaining populations of CD4⁺ and CD8⁺ T cells. Because larger numbers of CD8⁺ T cells are available to undergo expansion and IL-7 appears to expand CD8⁺ T cells preferentially compared with CD4⁺ T cells[55], substantial expansion of CD8⁺ T-cell populations would occur.

Lymphoproliferative disorders in TCD hosts

TCD hosts with HIV infection[56] and recipients of TCD bone-marrow transplants[57] are at a greatly increased risk of lymphoproliferative disorders or lymphoma. Although the uncontrolled replication of lymphotropic viruses owing to a loss of immune surveillance clearly plays a central role in this process, it is important to note that animals genetically engineered to have chronic elevations in the level of IL-7 also develop lymphoproliferation and/or lymphoma, independent of viral infection[58,59]. The mechanism of IL-7-induced transformation is not entirely known, but the up-regulation of expression of members of the bcl-2 family by IL-7 might play a role, because genetically induced elevations of the level of bcl-2 by the t(14;18) translocation are a crucial step in the development of follicular lymphoma[60]. Thus, combining uncontrolled viral replication with the effect of IL-7 on developing lymphocytes would be expected to heighten greatly the opportunity for lymphoproliferation and neoplastic transformation. Indeed, the capacity of exogenous IL-7 to induce lymphoma in TCD hosts will be one issue that must be addressed carefully as this agent is developed for clinical use.

Autoimmunity following T-cell depletion

As described previously, increased levels of IL-7 not only increase Ag-driven proliferative responses to high-affinity Ag but also, convert otherwise tolerogenic or nonimmunogenic Ags into proliferative stimuli (Fig. 1). Although this process might be helpful for maintaining repertoire diversity during the phase of T-cell regeneration, it could also predispose to a breakdown of self-tolerance and subsequent autoimmunity. Some experimental models that allow a clear distinction to be made between T-cell responses to high- versus low-affinity peptides have led to the conclusion that low-affinity Ags do not induce the development of effector cells, despite inducing proliferation[38,39]. However, in natural settings associated with T-cell depletion, it seems probable that the antigenic milieu present within a host will comprise an array of peptides with varying affinities, some at the extreme ends of high- or low-affinity – which fit nicely into cognate- or self-peptide categories, respectively – but many in an intermediate group. One could imagine that the recruitment of T cells specific for intermediate-affinity peptides into effector populations would benefit TCD hosts, because this would increase the clonal frequency of pathogen-specific cells. Simultaneously, however, the generation of effector cells specific for intermediate-affinity self peptides would place the host at risk of autoimmunity. Indeed, experimental and clinical evidence suggests that the fine line between improving immune competence and causing autoimmunity is a dangerous one in TCD hosts; several examples of autoimmunity associated with T-cell depletion have been reported[61,62]. Furthermore, in some autoimmune diseases, such as juvenile rheumatoid arthritis[63] and bullous pemphigoid[64], elevated circulating levels of IL-7 are observed. Thus, although the biological effect of elevating the level of

IL-7 in response to T-cell depletion is to potently enhance immune competence in TCD hosts, it is predicted that elevated levels of IL-7 might also predispose to autoimmunity in some situations. Whether the ability of IL-7 to break tolerance might be exploited in the context of vaccine trials for cancer and other diseases will be the basis for future studies.

Conclusions

IL-7 is well recognized as a crucial cytokine for the early development of lymphocytes. Recent work has illustrated that IL-7 modulates potently peripheral T-cell responses to cognate Ag *in vivo*[22] and that IL-7 is a nonredundant cytokine for the PHE that occurs in TCD hosts[6,7]. Furthermore, clinical studies of TCD humans show that circulating levels of IL-7 are elevated in response to T-cell depletion and return to normal upon recovery of T-cell populations[9–11]. Together, these data generate a model wherein IL-7 functions as a master regulator of T-cell homeostasis by becoming increasingly available following T-cell depletion. Presumably, the increased levels of IL-7 both enhance thymopoiesis and increase PHE, as mechanisms to restore immune competence in TCD hosts. Further studies are necessary to determine whether the increased levels of IL-7 result from diminished target-cell binding with a lack of utilization or reflect increased stromal-cell production.

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Gelatinase B: a tuner and amplifier of immune functions

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Gelatinase B (matrix metalloproteinase-9) is a secreted multidomain enzyme that is important for the remodeling of the extracellular matrix and the migration of normal and tumor cells. It cleaves denatured collagens (gelatins) and type IV collagen, which is present in basement membranes. In the immune system, this cleavage helps lymphocytes and other leukocytes to enter and leave the blood and lymph circulations. Gelatinase B also cleaves myelin basic protein and type II gelatins, and this clipping leads to remnant epitopes that generate autoimmunity, the so-called REGA model of autoimmunity. Recently, gelatinase B has been found to process cytokines and chemokines, resulting in skewed immune functions. Therefore, gelatinase B, often considered as a pure effector molecule, acts as a switch and catalyst in both innate and specific immunity, and constitutes a prototypic example of the regulation of immune functions by proteolysis.

Matrix metalloproteinases (MMPs), which have a zinc ion in their active site, are a class of secreted enzymes with major functions in the degradation and remodeling of all components of the extracellular matrix (ECM)[1]. Remodeling of the ECM does not consist simply of proteolysis for the elimination of proteinaceous debris. Instead, the actions of MMPs are important in the regulation of normal developmental processes and are involved in pathological conditions. In this way, MMPs resemble the caspases, which are another class of proteases known for their function in programmed cell death or apoptosis. Indeed, the first identified MMP, collagenase (MMP-1), was discovered as a key molecule in the metamorphosis of frogs, a well-investigated example of developmental processes[2].

Increased levels of various MMPs (collagenases, stromelysins and gelatinases) have been detected in and are associated with inflammatory diseases of connective tissues[3–5] and invasive cancer processes[6]. Therefore, many efforts have been made to search for inhibitors of MMPs. Four natural tissue inhibitors of MMPs (TIMP-1–TIMP-4) show limited specificity towards particular MMPs (Ref. 7). Any MMP activity in the circulation is also inhibited adequately by the broad-spectrum protease

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