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MHC-dependent survival of naïve T cells? A complicated answer to a simple question

Jeffrey R. Dorfman, Ronald N. Germain *

Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bldg. 10 Rm. 11N311, 10 Center Dr. MSC-1892, National Institutes of Health, Bethesda, MD 20892, USA

Abstract

The differentiation and survival of developing αβ thymocytes depends on effective T-cell receptor (TCR) signaling upon recognition of self peptide/major histocompatibility complex (MHC) molecule ligands. Although this concept is uniformly accepted with regard to immature thymocytes, there are conflicting reports as to whether or not MHC recognition is required for survival of mature peripheral naïve T cells. In this review, we assess these reports critically and conclude that in many cases, the differences observed in $CD4^+$ T-cell recovery between MHC-expressing and MHC-deficient animals can be attributed to proliferation occurring only in the MHC-expressing lymphopenic animals studied in these models systems, rather than to effects of MHC recognition on cell viability per se. Still other reports involve experimental manipulations that may have affected the intrathymic development of the T cells such that they receive a "poor" selecting signal, fail to fully mature, and thus behave more like thymocytes in their survival characteristics (i.e., show MHC dependence). With respect to $CD8^+$ T cells, we discuss data suggesting that some clones are more dependent upon the presence of MHC class I for survival than others. We propose that some $CD8^+$ T cells even in a wild-type host may behave like the manipulated $CD4^+$ T cells just described, and fail to mature completely with respect to their survival requirements. Although the proportion of $CD8^+$ cells in this MHC-dependent state is not known, the corresponding fraction among $CD4^+$ T cells seems to be rather small. Overall, our analysis of the available data suggests that most or all mature $CD4^+$ (and perhaps also many $CD8^+$) T lymphocytes do not depend on self-recognition for their viability in the periphery. Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Cell survival; T lymphocytes; Major histocompatibility complex

1. Introduction

The generation of mature $CD4^+$ and $CD8^+$ αβ T cells depends on effective T-cell receptor (TCR) signaling upon recognition of self peptide/major histocompatibility complex (MHC)-encoded ligands in the thymus. Thus, it is quite possible that encounters in the periphery with the molecules involved in positive selection might still affect survival or other functional characteristics of naïve T cells. Several reports describe such effects; for example, proliferation of naïve T cells in T-cell-deficient environments depends on the presence of particular MHC-peptide combinations [1–9]. Additionally, other studies have concluded that the survival of naïve T cells requires MHC molecule recognition [10–17]. Both of these effects have been attributed to recognition of the relevant selecting self peptides, although at least one report has suggested that cross-reactive environmental antigens are necessary for naïve cell division [4]. However, more recent reports using polyclonal T cells [18,19] have yielded results suggesting that the survival of naïve peripheral T cells does not require constant stimulation by TCR signals arising from MHC recognition.

Here, we critically review the data in the field with the intent of finding explanations consistent with as much of the published data as possible. We suggest that there are alternative explanations for all of the results interpreted as evidence that naïve mature $CD4^+$ T cells need to recognize MHC class II in order to survive and also for some of the data suggesting that $CD8^+$ T cells need to recognize MHC class I to maintain their viability. Most of the observations of differential $CD4^+$ T-cell recovery from animals with and without MHC class II expression can be attributed to proliferation occurring only in the MHC-expressing lymphopenic animals employed, rather than to effects of MHC recognition on cell viability per se. It is important to distinguish MHC-dependent proliferation from MHC-based

* Corresponding author. Tel.: +1-301-496-1904; fax: +1-301-496-0222.
E-mail address: rgermain@niaid.nih.gov (R.N. Germain).

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maintenance of the viability of individual cells, even if either process could maintain the overall size of the population of "naïve" T cells. We reach less definitive conclusions concerning whether or not MHC class I recognition is required for survival of $CD8^+$ T cells.

2. Better cell recovery: MHC-dependent proliferation or MHC-dependent survival?

As described above, naïve T cells proliferate in T-cell-deficient (i.e., lymphopenic or "empty") environments. This proliferation requires both lymphopenia and recognition of MHC molecules by the T cells [1–9]. The response is heterogeneous, in the sense that T cells bearing certain TCRs proliferate and others do not. Those that do divide require presentation of particular peptides by the corresponding MHC [3–9].

Many of the publications claiming that normal $CD4^+$ T-cell survival depends on MHC class II recognition involve conditions in which the cells in the selecting MHC^+ environment are likely to proliferate much more extensively than in the nonselecting or putatively MHC-deficient host. In contrast, the reports that show no effect of MHC expression on $CD4^+$ T-cell survival for at least one month [18,19] use mice in which such proliferation is minimized. Thus, rather than assessing only the relative rates of cell death in nondividing cell populations, which is what is necessary when considering the influence of MHC proteins on cell viability, the experiments in papers that argue for MHC-dependent survival actually compare the combined effect of death and proliferation in MHC^+ hosts to death alone in the MHC^- hosts (Table 1).

One clear example of such proliferation is found in the work of Kirberg et al. [14]. These investigators recovered 30 times as many cells as they injected into a lymphopenic ($RAG^{-/-}$) host expressing the selecting MHC allele. The authors transferred only 10^5 cells under conditions in which the expected engraftment is about 25% [19] to 40–50% [11,20]. It is thus not surprising that it was difficult for these investigators to find the 25,000–50,000 donor T cells (less any that died an MHC-unrelated death over 7 weeks) we estimate would be present in nonselecting recipients in which no expansion occurs.

The report by Brocker [13] is also consistent with this proliferation-based interpretation of cell recovery data from MHC class-II-expressing and nonexpressing hosts. This investigator placed MHC class-II-expressing thymic grafts under the kidney capsules of MHC class-II-deficient mice and found measurable numbers of $CD4^+$ T cells in the periphery only when the recipient expressed MHC class II on its dendritic cells. This led the author to conclude that recognition of MHC class II is required for survival of $CD4^+$ T cells emigrating from the graft. But an alternative explanation is likely. The small output of $CD4^+$ T cells from a thymic graft of 3×10^6 cells would make detection of these cells outside the thymus difficult without their proliferation, which would be expected to occur only when MHC class II

Table 1
A summary of the reports concluding that survival of naïve T cells requires MHC recognition. For each report, a brief description of the method is given, as well as what type of T cell was used and the genotype of the MHC-deficient environment (MHC-deficient host for cell transfer experiments). For perspective, the reported or deduced half-life of T cells in the MHC-deficient environment (where calculable) is given. In the last column, the reason we argue that survival differences were not measured is given

Reference	Model	Cell type	TCR tg or polyclonal T cells?	MHC of deficient host?	T-cell $t_{1/2}$ in MHC-deficient environment [a]	Difficulties with survival assessment
[10]	Thymic graft	$CD4^+$	Polyclonal	$A\beta^{-/-}$ $RAG2^{-/-}$	1–3 months[b]	Confounding proliferation
[11]	Cell transfer	$CD8^+$	HY TCR Tg and polyclonal	$D^{b-/-}$ and $D^{b-/-}$ $\beta2m^{-/-}$	~3 d	Did measure survival differences
[12]	Viral expression of thymic MHC II	$CD4^+$	Polyclonal	$A\beta^{-/-}$	3–4 weeks	No MHC^+ comparison; also may have been performed in the presence of MHC class II
[13]	Thymic graft	$CD4^+$	Polyclonal	$A\beta^{-/-}$	Below detection limit	Confounding proliferation
[14]	Cell transfer	$CD4^+$	AB II TCR Tg ($I\text{-}E^d$/HA111-9)	Allogeneic nonselecting	<7 weeks	Confounding proliferation
[15]	Thymic graft	$CD8^+$	Polyclonal	$\beta2m^{-/-}$	Below detection limit	Confounding proliferation
[16]	Cell transfer	$CD8^+$	HY TCR Tg	TAP-$1^{-/-}$ $RAG1^{-/-}$	~1 week	Either confounding proliferation or did measure survival differences
[17]	Tet regulatable MHC class II transgene	$CD4^+$	Polyclonal	$A\beta^{-/-}$	3–4 weeks	No appropriate MHC^+ comparison

[a] Reported value or approximate value deduced by us from the data given.
[b] Not corrected for proliferation.

is expressed in the periphery. The MHC class-II-deficient hosts employed are likely to have been sufficiently lymphopenic even with their normal numbers of $CD8^+$ T cells to induce some proliferation of $CD4^+$ thymic emigrants. Our studies [19] are consistent with this possibility, as we observed proliferation of transferred $CD4^+$ T cells in the same MHC class-II-deficient animals that have been contaminated with rare class-II-expressing cells. This proliferation is clearly dependent upon expression of MHC class II because it is blocked by anti-class II antibody [19]. Thus, mice that are genetically class-II-deficient are sufficiently lymphopenic to support some proliferation of $CD4^+$ T cells, as long as a few appropriate cells bearing MHC class II molecules are present.

Another report describes a similar effect of peripheral MHC class I expression on $CD8^+$ cell number following implantation of an MHC class-I-expressing thymic graft in $\beta 2m^{-/-}$ recipients [15]. These data were interpreted by the authors as evidence for MHC-dependent cell survival, but may also be reinterpreted in the same manner as the Brocker paper. We consider it likely that the thymus did not seed the periphery with a sufficient number of $CD8^+$ cells to be directly measured. $CD8^+$ cells may have become detectable only after homeostatic proliferation or foreign antigen stimulation of cell division following injection of syngeneic or allogeneic MHC class-I-expressing tumor cells, respectively.

We do not suggest that MHC-dependent proliferation is unimportant in maintaining naïve T-cell number. The steady-state number of naïve T cells is determined by a combination of input from thymic export plus proliferation and removal by death. We do argue that one should not confuse maintenance of T-cell number by proliferation with maintenance of cell number through effects on T-cell viability. It is mechanistically quite different to suggest that MHC has a role in keeping an individual T cell alive as opposed to controlling the number of progeny that cell may have.

A notable consequence of repopulating lymphopenic mice by naïve cell proliferation is the preferential expansion of particular clones, leading to a substantial skewing of the T-cell repertoire [21]. This is evident in the fact that some but not other TCR transgenic T cells proliferate in lymphopenic hosts, and those that do proliferate do so at differing rates [4–7,9]. Furthermore, cells that have undergone homeostatic proliferation often undergo a change in phenotype and responsiveness [22,23]. Thus, whether the MHC-dependent effect upon T-cell number is by differential proliferation only or includes effects on survival as well has implications for more than just the mechanism by which population size is controlled. It also matters for our understanding of how the naïve T-cell repertoire and naïve T-cell function may vary over the lifetime of the organism, especially after thymic involution.

3. Does all “homeostatic” proliferation require MHC expression?

Several studies reported proliferation of $CD4^+$ T cells in mice putatively lacking MHC class II expression [4,10,12,18]. In one case [10], normal thymic grafts were placed under the kidney capsules of $RAG^{-/-}$ or $RAG^{-/-}$ $A\beta^{-/-}$ recipients. Some of the thymocytes present in the grafts matured and migrated to the periphery, where they were tracked. Substantial proliferation was seen in all recipients; but the donor cell number declined in the $RAG^{-/-}$ $A\beta^{-/-}$ recipients after a few months. We consider it likely that some MHC class-II-expressing cells also emigrated from the thymic grafts and that the MHC class II expressed by these cells was sufficient to promote proliferation in the $RAG^{-/-}$ $A\beta^{-/-}$ recipients. Eventually, these donor class-II-expressing cells would die, leading to a situation in which donor cell number would no longer be maintained by proliferation in the face of a constant rate of MHC class-II-independent death. In agreement with this scenario, decreased proliferation of donor cells in the $RAG^{-/-}$ $A\beta^{-/-}$ recipients as compared to those in $RAG^{-/-}$ recipients was seen at later assay times in this model.

In other cases [4,18], purified $CD4^+$ T cells were adoptively transferred to normal or MHC class II-deficient recipients. It is difficult to be certain that a small number of MHC class-II-expressing cells or their MHC class II-negative precursors did not contaminate the donor cell population. Contaminating cells could stimulate some homeostatic proliferation even when present at vanishingly small numbers that are difficult to detect except by T-cell proliferation itself. In our adoptive transfer model [19], proliferation of $CD4^+$ T cells was noted in genetically MHC class II-deficient recipients and this proliferation, but not that of $CD8^+$ T cells, was prevented by chronic injection of anti-class II antibody. Nonetheless, class II-expressing cells were not detected by PCR or flow cytometry. This provides clear evidence that MHC class II-expressing cells present at a level not detectable by conventional analytic methods may induce proliferation in such animals. In another study [4], the irradiated recipients were more grossly lymphopenic, and a substantial fraction of the $CD4^+$ T cells (about 20%) divided at least once within 7 d of transfer. Nonetheless, whether or not the cells that divided were naïve and whether or not contaminating class II-expressing cells might have been present to induce proliferation is hard to ascertain. At least some evidence indicates that proliferation of memory cells is independent of MHC recognition [24]. On balance, therefore, it appears that most or all proliferation of $CD4^+$ T cells in lymphopenic mice requires the presence of MHC class II-expressing cells, consistent with our proposal that such cell division is the primary confounding feature of existing studies claiming a relationship between MHC class II expression and naïve $CD4^+$ T-cell viability.

4. Reduced T-cell lifespan: compared to what?

Rooke et al. [12] concluded that MHC class II recognition is required for T-cell survival. In the experiments described, MHC class II expression was induced in one lobe of the thymus of $A\beta^{-/-}$ animals by use of a suitably engineered adenoviral vector. The periphery was devoid of MHC class II expression based on the assays performed. These manipulated thymii generated mature $CD4^+$ T cells that were exported to the periphery. BrdU experiments revealed that the peripheral $CD4^+$ T cells were proliferating. After correcting for this proliferation, the authors determined that the $CD4^+$ T cells in question had a half-life of 3–4 weeks. It was proposed that this lifetime is shorter than the known lifetime of naïve peripheral T cells and that the "enhanced" rate of death was due to the absence of MHC class II. However, these investigators could not actually measure the lifespan of $CD4^+$ T cells in otherwise equivalent MHC-expressing animals, so this contention is speculative. Indeed, other groups have transferred polyclonal $CD4^+$ T cells into normal unmanipulated recipients and found a similar 3–4-week half-life for the T cells [18,19,25]. Furthermore, the fact that there was substantial proliferation among these $CD4^+$ T cells implies the presence of MHC class II-bearing cells in the periphery of these mice, perhaps on such a small number of cells as to be difficult to detect other than by an effect on cell division. A wealth of other studies indicate that MHC class II molecules must be present for proliferation of most naïve $CD4^+$ T cells in lymphopenic hosts (see above). If there are enough MHC class II-bearing cells to stimulate such proliferation, then one might also presume that there are enough MHC class II molecules available to influence survival, if this is a property of MHC recognition by $CD4^+$ T cells. Thus, the reported half-life in this study is likely to be that of $CD4^+$ T cells in the presence, not the absence, of MHC class II expression.

In a related study, Witherden et al. [17] examined $CD4^+$ T-cell number in $A\beta^{-/-}$ mice possessing a tetracycline-regulated MHC class II transgene expressed uniquely in cortical thymic epithelium. This transgene was expressed during early life and then it was shut off experimentally by administration of tetracycline; mature $CD4^+$ T cells previously exported from the MHC class II-expressing thymus into a putatively MHC class II-deficient periphery were subsequently tracked. After repression of thymic MHC class II expression, peripheral $CD4^+$ T cells disappeared with a half-life of 3–4 weeks, which is not different than the half-life of cells transferred to MHC-normal recipients [18,19,25] or cells generated in the Rooke et al. study [12], where the proliferation of the $CD4^+$ T cells may be taken as evidence for MHC class II expression in the periphery.

Witherden et al. did make an effort to define the lifespan of $CD4^+$ T cells by following the survival after thymectomy of hosts with normal MHC class II expression. As in other studies [26], the trauma of the surgery induces substantial T-cell death. The authors reported that half of the normal $CD4^+$ T-cell population was lost acutely following surgery. This, perhaps accompanied by the loss of thymic $CD8^+$ T-cell output, would decrease peripheral T-cell numbers in these animals, creating a lymphopenic environment. But as noted above, polyclonal $CD4^+$ T cells in such a situation will undergo MHC class II-dependent proliferation. Thus, the constant number of $CD4^+$ T cells seen in these animals after the initial post-surgical decline may not reflect a long lifespan of nondividing cells maintained by MHC class II recognition, as concluded by the authors, but rather, the balance between homeostatic expansion and cell death as in the other examples cited above. No BrdU experiments were performed in the thymectomized animals to check for proliferation, nor were the cells phenotyped for markers that change during homeostatic expansion (such as CD44). Even if the lifetime of $CD4^+$ T cells were to be reduced in these mice, it could plausibly be attributed to untoward effects on developing CD4 single positive thymocytes or their precursors (which require MHC class II for maturation) due to the poor expression of the transgenic MHC class II in the thymic medulla. Thus, the data in these two papers do not argue convincingly for shortened lifetimes for $CD4^+$ T cells in MHC class II-deficient mice.

5. Evidence that MHC class I recognition promotes survival of some $CD8^+$ T cells

Several papers have suggested a relationship between MHC class I recognition and naïve $CD8^+$ T-cell survival. One [15] of these studies involves conditions that lead to the same difficulty in distinguishing effects of MHC recognition on proliferation from those on survival of individual cells and is discussed above. The other two reports [11,16] use HY TCR transgenic T cells [27]. HY TCR-expressing cells that do not coexpress a second receptor involving an endogenous TCR α chain do not proliferate even in lymphopenic mice containing the selecting MHC allele [5,11,28]. HY transgenic T cells from mice on an $RAG2^{-/-}$ background thus will not proliferate in either the MHC^+ or the MHC^- recipient and experiments using cells from such mice [11] do not have the same problem in interpretation as those with polyclonal $CD4^+$ T cells. Unfortunately, one of the studies using cells with this TCR did not employ cells from $RAG^{-/-}$ or SCID background animals and is potentially flawed by the possibility of cell proliferation stimulated through a coexpressed endogenous TCR. No test for such proliferation was performed in this investigation [16], even though effort was made to exclude cells expressing endogenous receptors from the donor population.

This leaves one example involving the HY TCR in which variations in cell recovery in the selecting versus nonselecting MHC environment cannot be ascribed to differential proliferation. In this case, the difference in recovery was quite large, 30–50-fold different between the MHC class I-

expressing and class I-deficient recipients after 2 weeks [11]. It is noteworthy that the HY TCR is thought to have an unusually low avidity for its selecting ligand(s), raising the possibility that it is not representative of most $CD8^+$ T cells. This conclusion is based largely upon a series of studies that partially impaired the TCR signaling machinery and then studied how that affected thymic selection. Immature thymocytes bearing the HY TCR [29] but not the P14 TCR [30] show deficient positive selection when the immunoreceptor tyrosine-based activation motifs (ITAMs) are removed from or mutated in the TCRζ chain. Although these experiments were initially performed with different constructs of the mutant ζ chain in tests with each TCR, they were subsequently repeated with the same construct for both TCR [31]. Because the ligand needed for survival is often presumed to be the selecting ligand(s), we wondered if this might mean that the HY TCR transgenic T cells are not representative of polyclonal $CD8^+$ T cells in terms of the effect of MHC class I recognition on peripheral naïve cell survival. In preliminary cotransfer experiments of our own, we have determined that HY transgenic $RAG2^{-/-}$ T cells survive less well in $TAP1^{-/-}$ recipients than do naïve polyclonal $CD8^+$ T cells. Although the interpretation of these data is complicated by a concomitant slow rejection of all cells in this transfer combination, presumably mediated by the host $CD8^+$ T cells [32,33], this experiment suggests that HY T cells are more sensitive to a reduction in the level of MHC class I in their environment than most other $CD8^+$ T cells. In support of this contention, we note that HY $RAG2^{-/-}$ T cells disappear more quickly than polyclonal $CD8^+$ T cells do in irradiated $\beta2m^{-/-} \times D^{b-/-}$ recipients. At 1 week, 3% of engrafted HY T cells remain while an estimated 30% of engrafted polyclonal $CD8^+$ T cells remain under the same transfer conditions [11]. Interestingly, and in support of this idea, a targeted mutation removing the tyrosine of the CD3ε chain prevents the accumulation of HY TCR transgenic T cells but not P14 TCR transgenic T cells [34], raising the possibility that HY T cells are more sensitive to TCR-mediated survival signals than P14 T cells.

One possible interpretation for these data is that some T cells require MHC in order to survive while others do not. For example, strong TCR signals during thymic selection may be necessary for proper long-term expression of a receptor or signal transduction element needed for the T cell to take advantage of a non-TCR survival stimulus in the periphery. This proposal could explain why HY TCR transgenic T cells in particular appear to have a shortened lifetime in the absence of MHC and a lower steady-state number even in MHC-expressing animals. It leaves open the key question of whether the viability of the bulk of the $CD8^+$ T cells that appear less sensitive to the (partial) withdrawal of MHC class I truly reflects the lack of a requirement for MHC class I recognition for sustained survival or if this result reflects the capacity of these cells to get enough signal for survival from their fellows' normal levels of MHC class I and/or from the very little remaining MHC class I expressed in $TAP1^{-/-}$ and perhaps also irradiated $\beta2m^{-/-} \times D^{b-/-}$ mice. As far as we can see, there are no available data that would allow one to distinguish between these two possibilities.

We also need to point out that there are some questions as to whether the rapid loss of HY TCR $Rag2^{-/-}$ T cells in the cited study actually represents loss of viability due to a lack of MHC class I recognition or if rejection by the MHC class I-deficient host of the MHC class I-expressing transgenic T cells accounts for the findings. Rejection was discounted as an explanation for the data based on the persistence of MHC class I-expressing polyclonal $CD4^+$ T cells transferred into other $\beta2m^{-/-} \times D^{b-/-}$ recipients. But because these recipients were irradiated and thus lymphopenic, these $CD4^+$ T cells would proliferate and the maintenance of their number may have been a consequence of a balance of such proliferation and the concomitant rejection. The HY T cells, in contrast, would be lost rapidly because of a lack of the cell division needed to compensate for losses due to this rejection. Although several unpublished studies suggest that the rejection of class I normal cells by class I-deficient recipients is abrogated by irradiation, this type of logical error remains a potential pitfall in designing appropriate controls for survival experiments. This remains an issue of contention (see note 15 of Murali-Krishna et al. [24]).

6. Incomplete acquisition of the capacity for MHC-independent survival: a general phenomenon?

We have argued above that an insufficient signal during positive selection in the thymus might result in the failure of some maturing T cells to fully develop the capacity to survive efficiently using only non-TCR-related signals (e.g., those coming from stromal cytokines) in the periphery. Under such conditions, these cells may be able to derive some survival benefit from constant MHC-mediated stimulation, using signaling machinery retained from the immature thymocyte state when persistent MHC-derived input is known to support viability. Interestingly, some TCR transgenic T cells seem to have an impaired lifetime compared to other T cells even in the presence of MHC [35]. Perhaps this occurs if the signals received from MHC recognition in the periphery are less efficient at sustaining cell viability than the MHC-independent survival signals that typically perform this task. This may not only be true of specific T cells assessed in transgenic models but also in general for the helper-type lineage T cells in $CD4^{-/-}$ mice [36].

In normal mice, the proportion of such survival-defective T cells amongst $CD4^+$ must be low, at least among $CD4^+$ T cells. Were they to be a larger proportion of the $CD4^+$ T-cell pool, their absence in $A\beta^{-/-}$ recipients of transferred cells would have been noted previously [18,19]. Proliferation of "MHC-independent" T cells could not have compensated for dying "MHC-dependent" $CD4^+$ T cells in these experi

ments because donor cells that divided after transfer were always rare and often excluded from the analysis. For this reason, the maximum proportion of MHC-dependent T cells among polyclonal $CD4^+$ T cells is limited to the error of measurement in the experiment, perhaps 20–30%. However, because a great deal of evidence indicates that $CD8^+$ T cells receive less intense or shorter duration TCR signals during their development in the thymus [37], $CD8^+$ T cells as a group might be more akin to the rare inadequately matured $CD4^+$ T cells that seem to show some sensitivity to the presence of peripheral MHC molecules. Thus, a larger fraction of $CD8^+$ T cells could utilize post-thymic MHC recognition to enhance their survival. The available data do not permit us to assess whether this is the case.

7. Competition for niches and CFSE toxicity

The reports suggesting that $CD4^+$ T cells do not require recognition of MHC class II molecules for survival use B6 mice as control recipients [18,19]. It has been suggested that B6 mice are inappropriate controls because the $CD4^+$ T cells are lost in these hosts for different reasons than those causing their disappearance in $A\beta^{-/-}$ recipients. They are presumably lost in the $A\beta^{-/-}$ recipient as a result of neglect, whereas they are argued to be lost in the B6 recipient as a result of competition with host $CD4^+$ peripheral T cells and $CD4^+$ thymic emigrants [38].

Although the B6 recipients are an imperfect control, we consider this alternate explanation to be quite unlikely, as it does not fit well with our data. If competition with pre-existing $CD4^+$ T cells was having a substantial effect under these experimental conditions, then one might have expected a smaller initial engraftment or an early decline in transferred $CD4^+$ T-cell number in B6 compared to $A\beta^{-/-}$ recipients. Yet neither was the case. We further suggest that it is unlikely that the loss of transferred cells due to competition in normal mice would precisely match the rate of death by neglect and potential competition with host $CD8^+$ T cells in the MHC class II-deficient mice. Yet this would have to be true to explain the results. Investigators postulating this explanation for the intact animal transfer data also assume that naïve $CD4^+$ T-cell "niches" and naïve $CD8^+$ T-cell niches are distinct, but we are not aware of any data that address this properly. Data do exist on proliferation of $CD4^+$ and $CD8^+$ naïve T cells in lymphopenic mice and such expansion is affected by the presence of the other T-cell lineage [5,39]. As to the issue of thymic export, preliminary results indicate that transferred $CD4^+$ T cells survive equally well in thymectomized recipients that are B6- or class II-deficient (JRD and RNG, unpublished data).

Another potential artifact postulated to account for the similar survival of $CD4^+$ T cells in MHC class II-expressing and deficient mice is toxicity of the CFSE label used to track the donor cells and examine their division after transfer. Both of the studies claiming a similar lifespan for $CD4^+$ T cells in the presence and absence of MHC class II molecules use such labeled cells [18,19]. However, transfer experiments into normal mice using cells marked by Ly5 allelic differences revealed no effect of CSFE labeling on the lifetime of the $CD4^+$ T cells under our experimental conditions (JRD and RNG, unpublished data). Thus, the 3–4-week half-life of $CD4^+$ T cells in MHC class II-expressing hosts cannot be attributed to an artificially low estimate of the true lifespan due to dye toxicity. We also need to emphasize that this half-life matches that seen in others' transfer of normal T cells into normal recipients [25] as well as in the Rooke et al. study [12], which we suggest is also a measure of $CD4^+$ T-cell survival in the presence of MHC class II molecules, based on the documented proliferation of these lymphocytes in secondary lymphoid compartments.

8. Concluding remarks

The factors involved in controlling the population size and diversity of naïve T cells will determine the readiness of the organism to respond to pathogens to which the organism has not previously been exposed. Despite (or perhaps because of) the importance of these factors in T-cell biology, the recent literature has been contentious with respect to what is presumed to be a key survival signal for naïve T cells: TCR signaling upon self-MHC molecule recognition in peripheral lymphoid compartments. In this review, we have provided a critical reinterpretation of the existing published data, attempting to provide a coherent, consistent (if incomplete) explanation for seemingly divergent results and clearly divergent interpretations.

We conclude that the data interpreted as evidence that $CD4^+$ T cells need to recognize MHC class II ligands in order to survive may be explained by other phenomenon, particularly MHC class II-dependent proliferation that is now known to occur in the lymphopenic mice that were often used as models in these studies. Altered intrathymic selection of T cells or a failure to measure the corresponding T-cell lifespan under MHC-expressing conditions may explain the other reports dealing with this issue. With respect to $CD8^+$ T cells, the picture is more complex and less complete. Experiments using cells from a specific TCR transgenic (HY) support the view that at least some $CD8^+$ T cells require MHC class I-mediated recognition for optimal peripheral survival. It is possible that that $CD8^+$ T cells and $CD4^+$ T cells may be fundamentally different with respect to their survival requirements. Nonetheless, it is also possible that HY T cells are not a good model for the average $CD8^+$ T cell, which lives longer than an HY T cell in the same MHC class I-deficient environment. We suggest that due to the weak selection signal that promotes $CD8^+$ lineage commitment [37], a fraction of the $CD8^+$ T cells with the least avid TCRs will fail to differentiate past the stage of dependence on MHC survival signals normally seen among

thymocytes. HY may be a particularly good example of such a poorly selected $CD8^+$ cell, and a small minority of $CD4^+$ T cells might fall into the same category.

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