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Homeostasis and the age-associated defect of CD4 T cells

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Abstract

Survival and homeostatic division of naive CD4 T cells is regulated by the cellular and non-cellular milieu and together these processes ensure that a population of naive CD4 T cells persists into old age. However, the naive CD4 T cells from aged animals show reduced IL-2 production, proliferation, helper function and effector generation and memory function. We explore here whether the age-related defects in naive CD4 T cells are due to the aged environment from which they come or to intrinsic defects that are caused by homeostasis and their long lifespan.

Keywords

CD4 T cells; Aging; Immunization; Homeostasis

1. Age-related defects in immune function

Elderly humans are much more susceptible to infectious disease and experience increased morbidity and mortality when they become ill [1,2]. Current vaccines induce both reduced antibody (Ab) production and less vigorous cell mediated immune response in the elderly [3,4]. For example, Ab production in response to vaccinations for influenza, tetanus, hepatitis and *Streptococcus pneumoniae* are all reduced in the elderly [5-9]. These studies found that not only are the Ab levels reduced with age, the neutralizing and opsonizing function of these Abs is also greatly reduced. Age-related changes also result in decreased levels of somatic hypermutation of immunoglobulin genes following immunization [10-14], which is important for the production of highly effective antibody responses that can protect from infectious agents. Thus, reduction of these processes lead to reduced efficacy of vaccinations in the elderly, leaving them more susceptible to infections.

In most instances, the production of a protective Ab response requires the formation of germinal centers (GCs) in secondary lymphoid organs [15-17]. Germinal center development is critical, since it is within GC that responding B cells proliferate and differentiate into high affinity isotypeswitched plasma cells that are the hallmark of a protective Ab response. The formation of memory B cells is also GCdependent. The generation of GCduring an immune response is not yet fully understood but it is known to require antigen (Ag)-specific CD4 T cells, antigen-specific B cells and follicular dendritic cells. Using an adoptive transfer model, we have recently shown [18] that germinal center formation is significantly reduced when aged CD4 T cells are transferred into young immunized hosts. In contrast, when young CD4 T cells are transferred into aged hosts, germinal center formation is very similar to that found in young hosts. Our results strongly suggest that age-related defects in the cognate helper activity of CD4 T cells are responsible

for defects in germinal center formation, reduced antigen-specific B cell expansion and differentiation and reduced IgG production.

CD4 T cells are major players involved in responses to both infectious diseases and vaccinations. As mentioned above, they act as helpers, enabling B cells to differentiate into Ab secreting plasma cells, to isotype switch, to undergo somatic mutation and to become long-lived memory cells [19]. They also help CD8 T cells develop into cytotoxic cells [20] and are required for long-term CD8 memory generation [21,22]. Additionally, CD4 T cells mediate activation of macrophages, thus playing a critical role in viral [23,24] and bacterial [25] control. Thus, the focus of our studies has concentrated on how the function of CD4 T cells is influenced by aging. Other researchers have shown that T cell function exhibits significant age-related changes, including decreased in vitro proliferation, reduced graft rejection, reduced delayed hypersensitivity reaction and reduced tumor rejection [26]. These functional defects are likely the result of age-related changes in a multitude of signaling pathways in T cells including calcium mobilization, tyrosine phosphorylation [27-29] and NFAT and NFκB translocation [30,31]. We have shown that reduced NFκB translocation in naive CD4 T cells from aged mice is, at least in part, responsible for reduced in vitro and in vivo proliferation, as well as changes in cognate helper activity [30,32].

While the number of T cells in the periphery remains stable with aging [33], other changes do occur. One of the most dramatic changes in the peripheral T cell compartment with aging is the shift from a predominantly naive phenotype in the young to a predominantly memory phenotype in the aged [34-36]. This shift in phenotype is likely the consequence of the cumulative lifetime exposure to environmental antigens, pathogens and immunizations as well as to compensatory homeostatic proliferation in the periphery. Because of this phenotypic shift and the dramatic reduction in new T cell production with increasing age, only modest numbers of naive T cells are found in the periphery of aged individuals. Consequently, this and the age-associated defects in the response of the residual naive CD4 T cell population severely impairs the ability to respond to newly encountered antigens, including both pathogens and vaccinations, in the elderly.

2. A model to study aging of CD4 T cells

We have used a T cell receptor transgenic (TCR Tg) model for the majority of our studies. AND TCR Tg mice express a Vβ3/Vα11 TCR that is specific for a peptide of pigeon cytochrome c (PCC) presented on IE^k [37]. This model is very useful and allows us to directly compare naive CD4 T cells (CD44^{lo}CD62L^{hi}CD25^{neg}) from young and aged mice that have the same antigenic specificity [38]. One distinct advantage of this model is that it allows us to specifically examine naive T cells, thus eliminating one of the major issues in studying aging of the immune system, which is that memory phenotype T cells increase with age, as discussed above. In addition, this model allows us to compare young and aged TCR Tg CD4 T cells in both in vitro and in vivo situations and visualize their interactions with other cells within the immune system. In an extensive series of studies, we have found that a major defect in the immune response of aged mice is due to decreased intrinsic responsiveness of naive CD4 T cells from the aged animals [30,38-42]. In vitro and in vivo, aged naive CD4 T cells make less interleukin (IL)-2 and express lower levels of CD25 following TCR stimulation with antigen or plate bound antibody (either anti-CD3 or anti-Vβ3 with anti-CD28). Naive CD4 T cells from aged TCR Tg mice proliferate less vigorously following in vitro stimulation or immunization in an adoptive transfer model. Additionally, in vitro, the aged naive cells give rise to effector populations that are smaller, less differentiated and produce reduced levels of T helper type 1 or T helper type 2 polarized effector cytokines.

In vitro, the addition of IL-2, but not other common γ -chain-binding cytokines (IL-4, IL-7 and IL-15) restores expansion of aged CD4 T cells and leads to production of activated effector populations (rescued effectors). However, when such rescued effectors become memory T cells after transfer to adoptive hosts, these memory cells exhibit severely impaired responses (reduced proliferation, cytokine production and cognate helper function) upon restimulation, thus indicating the aging defect is heritable [40,43]. This result is in contrast to memory CD4 T cells generated using naive CD4 T cells from young mice (young effectors). When these young effectors are allowed to transition into memory cells and are then aged (1 year), they still exhibit a robust response to antigen ex vivo. They proliferate well in response to antigen and make high levels of polarized cytokines. Therefore, it is important to note that aging effects that influence the response of naive CD4 T cells do not have the same detrimental influence on memory CD4 T cells. Moreover, these results suggest that whatever mechanisms mediate the aging defect have distinct effects on T cells at different stages of differentiation. This finding is also encouraging since it indicates that immunologic memory generated during youth functions well into old age.

3. The effect of post-thymic lifespan on naive CD4 T cell function

Why does the defect in naive CD4 T cell function develop? In young mice thymic output of new naive T cells is high. As individuals age, the thymus involutes and by one year of age, is minimal. At the same time, the homeostatic mechanisms that maintain peripheral T cell numbers function to keep the numbers of cells relatively constant. This means that in a young mouse, the pool of naive CD4 T cells consists mostly of recent emigrants from the thymus. Since the total pool size remains roughly constant, the peripheral naive T cells must disappear at a rate which is equivalent to the output of new cells from the thymus. Thus, most of the naive T cells in young mice are themselves of short chronologic age. Even though thymic output becomes extremely low with aging, the total number of CD4 T cells in the periphery does not change dramatically and a cohort of naive CD4 T cells is maintained [33]. Thus, as the mouse becomes older, and the naive population shifts to one which consists of naive cells that have either been present for an extended time or are the progeny of such cells.

We have examined the fate of naive CD4 T cells with time, utilizing a transfer model in which young (or aged) naive CD4 T cells are labeled with the vital dye carboxy fluorescein succinimidyl ester (CFSE) and transferred to an intact host. When CFSE-labeled cells divide they partition the labeled proteins, so it is possible to visualize division and survival of undivided cells. Results indicate that the transferred cells divide infrequently (Haynes, Huston and Swain, unpublished and [44,45]), supporting the concept that the bulk of the naive population, present in older animals, consists largely of CD4 T cells which have a long chronologic age and have a long intermitotic lifespan as well. Therefore, we suggest that age-related defects in naive CD4 T cell function may be in part caused by the increased post-thymic “age” of the naive CD4 T cells in aged animals (Fig. 1).

To address the possibility that chronologic age is responsible for defects in naive CD4 T cells from aged mice, we have carried out a series of studies where we have manipulated the age of peripheral CD4 T cells experimentally [46]. In the first set of experiments, new T cells were generated by depleting CD4 T cells from the periphery of young and aged TCR Tg mice by anti-CD4 Ab treatment. After 8 weeks, the newly generated CD4 T cells were assayed for antigen-specific responses. Equal numbers of TCR Tg cells from each experimental group were stimulated with Ag and antigen presenting cells (APC). T cell proliferation over the 4-day culture period and IL-2 production was then examined. CD4 T cells from the anti-CD4 treated AND TCR Tg aged mice exhibited ex vivo responses similar

to young CD4 cells, while cells in the isotype treated aged group still exhibited significant age-related defects (Table 1).

Similar experiments were carried out using inbred B10.BR mice. After 8 weeks post-treatment with anti-CD4, mice were immunized with PCC conjugated to the hapten nitrophenyl (NP-PCC) and the number of responding NP-specific B cells was examined by flow cytometry. We have shown in previous studies that the cognate helper function of CD4 T cells severely declines with age and the expansion and differentiation of NP-specific B cells is CD4 dependent [18]. Table 1 shows that the aged B10.BR mice treated with anti-CD4 antibody exhibited cognate helper function similar to that seen in young mice, while aged mice in the control group displayed poor helper activity. These results show that newly generated CD4 T cells in aged mice respond robustly, both ex vivo and in vivo, in both TCR Tg and non-Tg models. This is exciting because the bone marrow and environment in the old mouse was able to give rise to thymic emigrants that were not demonstrably defective. It would be interesting to further examine whether newly generated CD4 T cells become defective at the same rate in young and older hosts, though the lifespan of the aged hosts may pose a limit on how long we can observe the development.

We next examined new T cells generated from young and aged bone marrow (BM). BM preparations from young and aged AND TCR Tg mice were transferred into lethally irradiated non-Tg B10.BR hosts to generate BM chimeras. After 12 weeks, recipients of both young and old BM had sizeable populations of BM-derived naive CD4 T cells and the ex vivo response of the newly generated TCR Tg CD4 T cells was examined. Both the levels of IL-2 secreted and the fold expansion over a 4-day culture period were determined. Table 2 shows that newly generated T cells from either young or aged BM responded well to antigen. When BM was transferred into either young or aged hosts, a similar pattern was seen and the newly derived naive CD4 T cells were apparently functional. This was in contrast to the peripheral TCR Tg CD4 T cells from the original aged BM donors (Table 2, lower portion), which exhibited significant reductions in both fold expansion and IL-2 production as we have shown previously [40]. These results suggest that newly generated TCR Tg CD4 T cells, even in an aged host environment, can respond well to antigenic stimulation. It should be noted that in the BM chimera experiments, the new thymic emigrants develop in a highly lymphopenic environment, and in all combinations presumably undergo homeostatic driven division (HDD). This may have some effect in normalizing their functions. Further studies will be needed to evaluate whether the HDD perturbs the function of the cells (see below).

In another set of experiments we endeavored to increase the chronologic age of the peripheral naive CD4 population by cutting off thymic emigration. AND TCR Tg mice were thymectomized (Tx) at 4 weeks of age. The ex vivo function of the Tg CD4 T cells was then examined at various time points after Tx. In the control sham treated mice, new T cells were still being generated throughout the experiment, while in the Tx mice, the cohort of T cells present at the time of surgery, became older in the absence of new T cell input. Table 3 shows that at 2 months post-Tx, there were no significant differences in the ex vivo function of Tg CD4 T cells from the two groups. Four months post-Tx, naive CD4 T cells from Tx mice had become slightly less functional. By 8 months post-Tx, significant decreases in both IL-2 production and CD25 expression were seen in the Tx group. Thus, by thymectomizing young mice and preventing new T cell production, we have induced acceleration in the appearance of an aged phenotype. A similar study in a non-transgenic model examining the effect of thymectomy on T cell function has been published previously with very similar conclusions [47].

Thymic involution may also contribute significantly to immunosenescence due to declines in T cell production and/or thymic hormone production [47]. A caveat of the Tx studies, is that the thymus may contribute hormonal or other factors that could impact T cells function. Thus, future studies will need to generate a population of chronologically old T cells by alternate strategies.

Taken together, the results of these experiments provide circumstantial evidence that an increase in the post-thymic age of naive CD4 T cells is associated with a decrease in their ability to respond to Ag. The first studies in CD4-depleted and BM chimeric mice also argue that the aged microenvironment has little impact, at least under the sets of circumstances used.

How would an increase in post-thymic age influence the response of CD4 T cells? One of the first events to occur following stimulation of a CD4 T cell by TCR ligation during interaction with an APC is the creation of an immune synapse. The formation of these plasma membrane microdomains increases the localization of TCR accessory signaling molecules and act as platforms for signal transduction [48]. Published studies have shown that naive CD4 T cells from aged TCR Tg mice do not form immune synapses with antigen presenting cells as efficiently as cells from young mice [31,49]. In addition, aging leads to reduced translocation of TCR-associated proteins to the immune synapse in these CD4 T cells. These age-related changes, which seem to originate at the cell membrane during synapse formation, result in the quality of the initial TCR signal being reduced in these cells, leading to many down-stream reductions in the signaling cascade. Changes in the cell membrane of older CD4 T cells in the aged animals are quite possible and could be due to a multitude of factors including oxidative stress, which has been shown to lead to reduced IL-2 production by naive, but not memory, CD4 T cells [50].

4. Factors that regulate naive CD4 T cell lifespan

Since the post-thymic age of CD4 T cells seems to dramatically influence their response to antigen, we have examined factors in the peripheral immune system that may regulate the lifespan of naive CD4 T cells. Although there is extensive literature examining the lifespan of CD4 T cells, the results are quite divergent, with different approaches yielding conflicting results.

Early studies of naive CD4 T cell lifespan involved Tx to prevent the production of new T cells along with enumeration of naive phenotype cells [51-54]. Results of this type of experiment, whether carried out in a normal inbred or TCR Tg models, show that some CD4 T cells decline rapidly, while others persist for many months. We have concluded that the lifespan of individual T cells varies over a wide spectrum and may depend upon regulatory events that come into play either with time and/or with loss of CD4 T cells and other environmental changes. One confounding factor with this kind of analysis is that T cells can convert to a memory phenotype either because of exposure to Ag or because they undergo HDD [55,56].

More recently, adoptive transfer experiments, which follow a cohort of naive cells after introduction into an adoptive host, have provided a more definitive model for examining lifespan and analyzing components that influence it. HDD, monitored by loss of CFSE dye label, and survival of donor naive CD4 T cells was examined in a variety of models. Interestingly, the greatest level of donor cell survival was found to occur in a lymphopenic environment, where there were no competing CD4 T cells, and sufficient MHC Class II was present (see Table 4).

Thus, one important factor in the regulation of naive CD4 T cell lifespan is the presence of CD4 T cells and possibly other cell types. With increasing age, the presence of memory phenotype CD4 T cells increases. In addition, in older individuals T cell clonal expansions (TCEs), which also express a memory phenotype, become more prevalent. TCEs (both CD4 and CD8) are very common in both mice and humans and often result from clonal expansions generated during chronic infections, such as cytomegalovirus, Epstein-Barr virus and varicella [57]. These TCEs are usually non-responsive and can take up significant space in the periphery. Our results show that the presence of other memory phenotype CD4 T cells in hosts significantly inhibits both the survival and HDD of transferred naive CD4 T cells (Table 4). It is possible that these memory phenotype CD4 T cells compete for survival factors or contain populations of regulatory cells that influence the survival of the donor CD4 T cells. Interestingly, the presence of naive or memory CD8 T cells has no effect on donor CD4 T cells (Table 4). The mechanism(s) by which such regulation occurs are currently undefined.

One additional factor that is vital for the survival of naive CD4 T cells is IL-7. The IL-7 receptor is expressed on resting CD4 and CD8 T cells and is responsible for providing signals that promote survival of the resting cells, such as upregulation of Bcl-2 expression [58]. Importantly, it has been shown that levels of IL-7 in the serum of older humans is significantly reduced compared to younger individuals [59]. Therefore, not only are the numbers of new T cells produced in the elderly decreased, important survival factors are also decreased. This is critical since our experiments and those of others [60,61] have shown that IL-7 is essential for the survival of naive CD4 T cells. Table 4 reflects experiments we have done in which donor TCR Tg CD4 T cells lacking IL-7R expression survive adoptive transfer much less well than do WT TCR Tg cells.

5. Impact of HDD on naive CD4 T cell function

Homeostasis of naive T cells is quite complex. Under normal conditions, the size of the naive T cell population is regulated by thymic output, by the longevity of the peripheral naive CD4 T cells and perhaps by their division. Several interacting factors are required for division due to lymphopenia including proper MHC Class II/peptide expression and availability of IL-7 [62-64]. When there is a lymphopenic environment, naive T cells undergo HDD and may achieve a memory phenotype [65]. This process may maintain naive cells and expand the possible memory T cell repertoire so that it is diverse enough, especially in neonatal animals, to respond to a broad range of pathogens. Since thymic output continues to decrease with aging, there may be more space available in the naive CD4 T cell niche of older animals compared to younger individuals, thus leading to a somewhat lymphopenic environment. We hypothesized that if this were the case and older CD4 T cells had undergone more cumulative division, this might have a negative impact on their function. Therefore, we examined the effect of HDD on the ability of naive CD4 T cells to respond to antigen using an adoptive transfer model. Naive TCR Tg CD4 T cells were transferred into hosts where they either could or could not undergo HDD. In adult thymectomized bone marrow reconstituted (ATxBM) hosts, the peripheral T cell compartment is empty but there is ample MHC Class II expression, allowing for HDD to occur. In MHC Class II KO hosts, the peripheral CD4 compartment is empty and there is no Class II expression, thus little HDD is observed. After 7 days, the transferred cells were recovered and tested for their ability to respond to antigen *ex vivo*. Table 5 shows that those cells that had undergone HDD responded poorly, while those that did not divide responded well. These results indicate that HDD may have a negative influence on the function of naive CD4 T cells and that as cells age, accumulated divisions might contribute to decreased CD4 T cell function with aging.

6. Summary and speculation

As individuals age, naive CD4 T cell function declines, leading to a reduced ability to respond to newly encountered pathogens and immunizations. We hypothesize that this defect in CD4 T cell function is due to multiple factors some of which are associated with an increased post-thymic age of the cells in older individuals. Using a murine model, we have shown that newly generated CD4 T cells in older animals function well, suggesting that the aged environment does not have a dominant detrimental influence. Additionally, we have shown that a number of factors that can negatively or positively influence the lifespan of naive CD4 T cells. Lifespan is increased by IL-7 and by MHC Class II/self-peptide recognition and decreased by memory phenotype CD4 T cells (Fig. 2) and these same factors regulate HDD as well. Thus, it is possible that the cohort of naive T cells that somehow survives in aged animals has been exposed to such signals repeatedly and these cells may in addition have undergone HDD. Perhaps this chronic exposure suboptimal activation signals contributes to their decreased responsiveness as shown in Fig. 2.

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Abbreviations

TCR Tg	T cell receptor transgenic
Ag	antigen
Ab	antibody
PCC	pigeon cytochrome <i>c</i>
NP-PCC	PCC conjugated to the hapten nitrophenyl
Tx	thymectomized
ATxBM	adult thymectomized bone marrow reconstituted
TCEs	T cell clonal expansions
IL	interleukin
APC	antigen presenting cells
CFSE	carboxy fluoroscein succinimidyl ester
GC	germinal center
HDD	homeostasis driven division

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Fig. 1.

Model for how defects in naive CD4 T cell function occur with increasing age. In young animals, thymic output is high, leading to a rapid turnover of T cells in the periphery. As animals age and thymic involution occurs, production of new T cells declines. Since the number of T cells in the periphery remains fairly constant, turnover of T cells in the periphery of aged animals must decline. This would result in T cells in the periphery of aged animals being chronologically older than those in younger animals.

Fig. 2.

Hypothetical impact on aging. Repeated signaling by TcR during recognition of self-peptide/Class II, without costimulation, may lead to loss of function over time, even if some such triggering contributes to survival. Loss of thymic hormones or reduction of IL-7 levels and negative homeostatic regulation by memory CD4 T cells could also cumulatively impact naive CD4 T cells. Finally homeostasis driven division, that seems to occur infrequently, might further contribute to loss of function. Loss of function is indicated by naive CD4 T cells becoming lighter as they age.

Table 1

Responses following anti-CD4 treatment of young and aged mice

Mice ^a	Treatment	T cell proliferation ^b	IL-2 production ^c	Helper function ^d
Young	Anti-CD4	++++	++++	++++
	Isotype	++++	++++	++++
Aged	Anti-CD4	++++	++++	++++
	Isotype	++	++	++

^a Young and aged TCR Tg mice were treated with anti-CD4 or isotype control antibody. After 8 weeks, CD4 cells were purified from spleen and LNs and stimulated in vitro with peptide Ag and APC.

^b T cell proliferation was determined by the total number of blasts recovered after 4 days of culture with Ag/APC.

^c IL-2 production after 24 h of culture with Ag/APC was determined by collecting supernatants and assaying for IL-2 in a bioassay.

^d Young and aged B10.BR mice were treated with anti-CD4 or isotype control antibody. After 8 weeks, mice were immunized with NP-PCC/alum. The number of responding NP-specific B cells was determined by flow cytometry.

Table 2

Responses following BM reconstitution of young and aged hosts

Tg BM donor ^a	Inbred host	T cell proliferation ^b	IL-2 production ^c
Young	Young	++++	++++
Aged	Young	++++	++++
Young	Aged	++++	++++
Intact Tg mice ^d			
Young		++++	++++
Aged		++	++

^a BM from young or aged TCR Tg donors was transferred to lethally irradiated young or aged B10.BR hosts. After 12 weeks, CD4 cells were purified from spleen and LNs and stimulated in vitro with peptide Ag and APC.

^b T cell proliferation was determined by the total number of blasts recovered after 4 days of culture with Ag/APC.

^c IL-2 production after 24 h of culture with Ag/APC was determined by collecting supernatants and assaying for IL-2 in a bioassay.

^d Purified CD4 T cell populations from young and aged TCR Tg mice were assayed for proliferation and IL-2 production in response to Ag/APC.

Table 3

Responses following thymectomy

Months post-Tx ^a		IL-2 production ^b	CD25 expression ^c
2	Tx	++++	++++
	Sham	+++	++++
4	Tx	+++	++
	Sham	+++	++++
8	Tx	+	+
	Sham	+++	+++

^aYoung adult TCR Tg mice were thymectomized at 4 weeks of age. After the indicated period of time, CD4 cells were purified from spleen and LNs and stimulated in vitro with peptide Ag and APC.

^bIL-2 production after 24 h of culture with Ag/APC was determined by collecting supernatants and assaying for IL-2 in a bioassay.

^cCD25 expression was examined after 4 days of culture by flow cytometry.

Table 4

Influence of environment on naive donor CD4 T cells

Host environment ^a	Relative survival	Relative HDD
Intact	—	—
Depletion of CD4 T cells	▲ ▲ ▲	▲ ▲ ▲
Class II deficiency	▼ ▼	▼ ▼ ▼ ▼ ^b
Depletion of CD8, Class I deficiency	—	—
Lack of IL-7	▼ ▼ ▼	▼ ▼ ▼ ▼
Memory phenotype CD4 T cells	▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ^c
Other naive CD4 T cells	—	—

^aNaive TCR Tg CD4 T cells were labeled with CFSE dye and transferred into a series of different adoptive hosts. At specific time points, the survival of the donor cells was monitored by determining donor undivided CD4 T cell recovery. HDD was determined by examining the CFSE profiles of the donor cells using flow cytometry.

^bClass II is required for the great majority of HDD.

^cEffects on HDD may include an effect that is clonotype specific [65]

Table 5

Impact of HDD on naive CD4 T cell responsiveness

Level of in vivo HDD ^a	T cell proliferation ^b	IL-2 production ^c
2+ divisions	+	+
No divisions	+++	+++

^a Naive young TCR Tg CD4 T cells were CFSE labeled and transferred to ATxBM or MHC Class II KO hosts. On day 7, donor cells were recovered by FACsorting. Populations were divided into a group that did not undergo any division and a group that exhibited two or more divisions based on CFSE profiles.

^b T cell proliferation was determined by the total number of blasts recovered after 4 days of culture with Ag/APC.

^c IL-2 production by 4-day effectors was assessed by intracellular staining following overnight restimulation.