Published in final edited form as:

Nat Rev Immunol. 2005 August ; 5(8): 655–660. doi:10.1038/nri1665.

Two sides of a cellular coin: CD4⁺CD3[−] cells regulate memory responses and lymph-node organization

Peter J. L. Lane, Fabrina M. C. Gaspal, and Mi-Yeon Kim

Peter J. L. Lane, Fabrina M. C. Gaspal and Mi-Yeon Kim are at the Medical Research Council Centre for Immune Regulation, Birmingham Medical School, Vincent Drive, Birmingham B15 2TT, UK.

Abstract

We propose that CD4⁺CD3[−] cells have two functions: a well-established role in organizing lymphoid tissue during development, and a newly discovered role in supporting T-cell help for B cells both during affinity maturation in germinal centres and for memory antibody responses. As CD4⁺CD3[−] cells express the HIV co-receptors CD4 and CXC-chemokine receptor 4, we think that infection of these cells by HIV, and their subsequent destruction by the host immune system, could help to explain the loss of memory antibody responses and the destruction of lymphoid architecture that occur during disease progression to AIDS.

A key feature of the immune system of both birds and mammals is that infection with pathogens elicits (within weeks) class-switched antibodies of precise specificity. This process occurs in germinal centres (GCs), which are potent advertisements for the power of natural selection. The generation of millions of B-cell-receptor specificities in the rapidly proliferating B-lineage centroblast population is followed by the ruthless selection of B cells that display these receptors by antigen and follicular T cells in an iterative cycle. At the end of this process, the affinity for antigen of the remaining B cells can be 10⁶-fold higher. It is also important that B-cell progeny survive as memory cells, which can be stimulated by re-exposure to small amounts of antigen to make rapid memory responses that eliminate the pathogen without signs of re-infection. All of this depends on the collaboration of T cells: follicular T cells help and select B cells during the crucial phase of affinity maturation in the GC, and memory T cells support memory B-cell antibody responses.

In this article, we propose a role for CD4⁺CD3[−] accessory cells in promoting the survival of T cells during both of these phases of the immune response. Furthermore, we suggest that these CD4⁺CD3[−] accessory cells are the adult equivalent of the inducer cells that organize the development of lymphoid tissue¹ and that these two functions — the organization of lymphoid tissue and the promotion of the survival of T cells that help B cells — are regulated by discrete sets of tumour-necrosis factor (TNF)-family members. Because of their expression of CD4 and CXC-chemokine receptor 4 (CXCR4), these accessory cells are potential targets for HIV, and we speculate that the loss of these cells is responsible for the loss of antibody responses and for the disorganization of lymphoid tissue that is observed with disease progression to AIDS.

Correspondence to P.J.L.L. e-mail:p.j.l.lane@bham.ac.uk.

Competing interests statement

The authors declare no competing financial interests.

CD4⁺CD3⁻ cell interactions with T cells

Digital confocal microscopy of spleen sections allowed the identification of interactions between T cells in GCs and a CD4⁺CD3⁻ cell population with dendritic morphology that lacks the mouse dendritic cell (DC) marker CD11c². CD4⁺CD3⁻ cells could be found not only in the B-cell follicles and GCs, where they were interacting with follicular T cells, but also aligned at the point where a B-cell follicle and the T-cell area adjoin (that is, the B–T interface), which is a site of T-cell–B-cell collaboration in both primary and secondary antibody responses^{3,4} (fig. 1). The priming of marked transgenic CD4⁺ T cells was mediated first by the conventional DC population in the T-cell area, after which some of the CD4⁺ T cells migrated to the outer T-cell area and into B-cell follicles, where a substantial proportion of them were found to interact with CD4⁺CD3⁻ cells².

Owing to the technical difficulties that are involved in isolating CD4⁺CD3⁻ cells from mice with an intact CD4⁺ T-cell repertoire, these cells were first isolated from the spleens² and lymph nodes (M.-Y.K. and P.J.L.L., unpublished observations) of T-cell-deficient mice. Two CD4⁺ cell populations could be distinguished from CD11c⁺ DCs: one was B220⁺CD11c^{low} and was identified as the plasmacytoid DC population⁵, and the other was CD4⁺CD3⁻CD11c⁻B220⁻ interleukin-7 receptor (IL-7R)⁺ (REF. 2). Humans also have a distinct CD4⁺CD3⁻ cell population in GCs⁶. Although the human cells express CD11c, they also express CD4, and their location is the same; therefore, we consider them to be equivalent to the mouse CD4⁺CD3⁻ cell population. In contrast to the DC-restricted expression of CD11c in mice⁷, CD11c is widely expressed by various lymphocytes and accessory cells in humans, and it is not a DC-specific marker⁸.

Signals to T cells from CD4⁺CD3⁻ cells

Because of the close association of the CD4⁺CD3⁻ cells with primed T cells, both in B-cell follicles (follicular T cells) (fig. 2) and at the B–T interface (newly primed and recirculating memory T cells) (fig. 3), it seems plausible that CD4⁺CD3⁻ cells provide co-stimulatory signals to T cells.

Unlike conventional DCs — which express, and can be activated through, CD40 — CD4⁺CD3⁻ cells (both mouse and human) have low levels of CD40 expression². Furthermore, their expression of conventional DC-associated co-stimulatory molecules, such as CD80 and CD86, is low, and we found no evidence that these cells could induce the proliferation of naive T cells². However, two T-cell co-stimulatory molecules are expressed at high levels by CD4⁺CD3⁻ cells: OX40 ligand (OX40L; also known as TNFSF4) and CD30 ligand (CD30L; also known as TNFSF8). These are both members of the TNF family, the receptors for which — OX40 (also known as CD134) and CD30, respectively — are expressed by primed, but not naive, T cells. In the context of the GC environment, OX40L can also be expressed by activated B cells⁹. Uniquely, however, the expression of both OX40L and CD30L by CD4⁺CD3⁻ cells is high and constitutive; it is independent of antigen activation and unmodified by exposure to cytokines, particularly IL-4 (REF. 10). Also, it has been shown by histological studies that there is membrane contact between OX40^{hi}CD4⁺ T cells and OX40L⁺CD4⁺CD3⁻ cells, which would allow the delivery of a signal through OX40 to the primed T cells².

Effects on T-cell function

OX40 and CD30 (the receptors for ligands that are expressed by CD4⁺CD3⁻ cells, OX40L and CD30L) are genetically linked in a TNF receptor (TNFR) cluster that has seven members and is located on mouse chromosome 4 and human chromosome 1. Similar to many other members of the TNFR family, they have common signalling pathways: both

bind members of the TNFR-associated factor (TRAF) family (TRAF1, TRAF2, TRAF3 and TRAF5)^{11,12}, and signalling through OX40 has been shown to upregulate the expression of anti-apoptotic B-cell lymphoma 2 (BCL-2)-family members, which promote survival¹³. Given that activated T cells can express both of these receptors, and that CD4⁺CD3⁻ cells express both of the ligands for these receptors, it is expected that there is partial redundancy between OX40 and CD30 signalling, and this has been shown by results from mice that are deficient in one or both of these receptors¹⁴.

T-helper-2 cell survival *in vitro*

CD4⁺ T cells that are activated *in vitro* upregulate the expression of both OX40 and CD30, but these receptors are expressed at a particularly high level at the surface of CD4⁺ T cells that have differentiated into T helper 2 (T_H2) cells after culture in the presence of IL-4 (REF. 2). Although many studies have associated OX40 and CD30 signalling with preferential differentiation into T_H2 cells *in vitro*, CD4⁺ T cells that are deficient in OX40 and/or CD30 can still proliferate and differentiate into T_H2 cells^{2,14}. The most important difference that these signals make is to the survival of T_H2, but not T_H1, cells. The co-culture of normal T_H2 cells with CD4⁺CD3⁻ cells shows independent additive effects of OX40 and CD30 signalling on T_H2-cell survival¹⁴.

Consistent with the survival effects of OX40 and CD30 signalling *in vitro*, an *in vivo* analysis of the immune response of mice that were deficient in both OX40 and CD30 showed independent and additive effects of these proteins on two aspects of T-cell help for B cells¹⁴: affinity maturation, and memory T-cell help for secondary antibody responses.

Affinity maturation supported by follicular T cells

The survival of the follicular T cells that orchestrate the affinity maturation of GC B cells depends on additive signals from CD30 and OX40 (REF. 14). Transgenic T cells that were deficient in both CD30 and OX40 initially proliferated normally, but similar to their *in vitro* counterparts, they failed to survive. As a consequence, although T-cell help for primary antibody responses was normal and GC development was initiated, the GCs involuted after 7 days. GC failure led to impaired affinity maturation of antibody responses. CD4⁺ T cells that were deficient in both OX40 and CD30 had restricted survival in B-cell follicles *in vivo*, which indicates that OX40 and CD30 have overlapping roles in the maintenance of follicular T cells¹⁴. These data support the *in vitro* observation that the role of the signalling through OX40 and CD30 that is induced by CD4⁺CD3⁻ cells (which express OX40L and CD30L) is to keep follicular T cells alive¹⁴.

It is clear that follicular T-cell survival is important for the affinity maturation of B-cell receptors in GCs. As affinity maturation progresses, the antigen that is driving the reaction becomes scarce; it is removed by phagocytes and masked by secreted antibodies. At this time, the follicular T cells are required for the selection of rare B-cell mutants with high-affinity receptors. Rapidly proliferating GC B-lineage centroblasts exit the cell cycle and differentiate into centrocytes, which compete for antigen fragments that are trapped at the surface of follicular DCs. Successful B-cell mutants that have high-affinity receptors can internalize antigen and successfully present peptide fragments to follicular T cells, which then provide selective CD40L-dependent rescue signals to the B cell. The constitutive expression of OX40L and CD30L by CD4⁺CD3⁻ cells, irrespective of the antigen concentration, allows these cells to provide antigen-independent survival signals to follicular T cells so that they can continue to select B cells (fig. 2).

T-cell help for memory antibody responses

Memory antibody responses in mice that are deficient in both OX40 and CD30 are markedly reduced¹⁴. By contrast, defects in memory antibody responses in mice that are deficient in either OX40 or CD30 alone are sufficiently mild to be ignored by many investigators who have studied OX40-deficient mice^{15–18} or CD30-deficient mice^{19,20}.

Memory T cells that provide help to memory B cells recirculate through lymphoid organs²¹, which ensures that the production of memory antibody responses does not depend on the site of initial immunization. The effects of OX40 and CD30 deficiency on memory antibody responses indicate that CD4⁺CD3[−] cells might specifically influence the survival of these memory T cells, as well as the follicular T cells that are involved in affinity maturation. Evidence from mouse studies indicates that recirculating memory T cells do not arise from the CXCR5-expressing follicular T-cell population that is responsible for affinity maturation in GCs, which does not recirculate²². A subset of memory T cells that home to the lymph nodes expresses both CXCR5 and CC-chemokine receptor 7 (CCR7)²³. The co-expression by B cells of CCR7 (the ligands for which are produced by stromal cells in the T-cell area²⁴) and CXCR5 (the ligand for which is produced by stromal cells in the B-cell areas²⁵) results in the alignment of B cells at the B–T interface²⁶; this also seems to be the case for the CXCR5⁺CCR7⁺ memory T cells in mice (R. Forster, personal communication).

In addition to being located adjacent to follicular T cells in B-cell follicles, CD4⁺CD3[−] cells are also found at the B–T interface². Although reagents are not available to assess expression of CCR7 at the surface of mouse cells, there is evidence that *Ccr7* mRNA is expressed by CD4⁺CD3[−] cells at the B–T interface (M.-Y.K. and P.J.L.L., unpublished observations). So, similar to their B-cell and T-cell counterparts, there are probably two subsets of CD4⁺CD3[−] cells: a CXCR5⁺ subset that is localized in B-cell follicles and interacts with CXCR5⁺ follicular T cells, and a CXCR5⁺CCR7⁺ subset that is present at the B–T interface and interacts with recirculating CXCR5⁺CCR7⁺ memory T cells.

Recirculating memory T cells do not usually express either OX40 or CD30, but they can be induced to re-express OX40 (REF. 14) by exposure to IL-7, which has been implicated in the maintenance of CD4⁺ memory T cells^{27–29}. We think that memory T cells that co-express CXCR5 and CCR7 upregulate OX40 in response to signalling mediated by IL-7 (perhaps produced by stromal cells or follicular DCs³⁰) as they migrate through secondary lymphoid organs; this allows them to receive OX40-dependent survival signals from CD4⁺CD3[−] cells each time they pass through lymphoid tissue (fig. 3). T-cell-dependent memory B-cell responses are therefore also promoted by the CD4⁺CD3[−] cell-mediated survival of memory T cells.

Neonatal CD4⁺CD3[−] cells

In contrast to CD4⁺CD3[−] cells from adult mice, CD4⁺CD3[−] cells that were isolated from neonates lacked expression of the molecules that are associated with T-cell memory, OX40L and CD30L, which indicates that the expression of these molecules is developmentally regulated³¹. Their absence is specific: the expression of ligands of the TNF family that are associated with the development of lymph nodes — such as lymphotoxin- α (LT- α ; also known as TNFSF1), LT- β (also known as TNFSF3) and TNF-related activation-induced cytokine (TRANCE; also known as TNFSF11) — is similar in adult and neonatal CD4⁺CD3[−] cell populations². This might help to explain the observations of Medawar and colleagues³², who reported 50 years ago that the immunization of neonatal rodents resulted in tolerance rather than immunity. We propose that T-cell help for B-cell responses is effectively aborted in neonatal rodents as a consequence of the absence of T-cell-survival signals through OX40 and CD30 that are initiated by CD4⁺CD3[−] cells in adults. This does

not render neonates immunodeficient, because they are protected by maternal antibodies. By the time of weaning, the levels of expression of OX40L and CD30L by CD4⁺CD3⁻ cells are similar to those in adult mice³¹, and the mice become competent to respond to infectious challenges.

Lymph-node organogenesis

Detailed phenotyping of adult CD4⁺CD3⁻ cells has shown that they are similar to a CD4⁺ cell population that was first described (by Mebius and colleagues³³) to colonize the lymph nodes of neonatal mice and was subsequently found in the spleen of neonatal mice³⁴. The name 'inducer' was later coined for these cells because of their essential role in the induction of lymph nodes, Peyer's patches¹ and, as has more recently been reported, isolated lymphoid follicles in the submucosa³⁵.

The splice variant of the retinoic-acid-receptor-related orphan receptor- γ t (ROR- γ t) is expressed by inducer cells and is required for their function in the induction of lymph nodes^{36,37}. Using a green-fluorescent-protein marker to identify ROR- γ t-expressing cells, Eberl, Littman and colleagues³⁵ reported a cellular phenotype that is similar to that of the CD4⁺CD3⁻ cells that we have identified in adults². Evidence from adult² and neonatal^{33–35,38} mice indicates that the shared phenotype of these CD4⁺CD3⁻ populations is as follows: CD4⁺CD3⁻ROR- γ t⁺CD45⁺LT- α ⁺LT- β ⁺TRANCE⁺KIT⁺IL-2R α ⁺IL-7R α ⁺ common cytokine-receptor γ -chain (γ c)⁺ CXCR5⁺CCR7⁺ α ₄ β 7⁻ integrin⁺THY1.2⁺CD8⁻CD11b⁻CD11c⁻B220⁻ natural-killer-cell 1.1 (NK1.1)⁻. We have found that mRNA encoding ROR- γ t is expressed by adult CD4⁺CD3⁻ cell populations, albeit at lower levels than in neonates, and adult and neonatal CD4⁺CD3⁻ cells share a distinctive pattern of immunity-associated gene expression that is different from that of conventional DCs (M.-Y.K. and P.J.L.L., unpublished observations).

The expression of TNF-family members that are associated with the development and organization of lymphoid tissue¹ — that is, LT- α , LT- β and TRANCE — is also common to both adult and neonatal CD4⁺CD3⁻ cells. In addition to being important for lymph-node development, there is evidence that expression of the LT- β -receptor (LT- β R) ligands, LT- α and LT- β , by neonatal inducer cells is responsible for the segregation of B cells and T cells in the developing lymph nodes³⁹. We think that CD4⁺CD3⁻ cells carry out a similar function in adults. The evidence for a non-lymphoid cell type (such as CD4⁺CD3⁻ cells) organizing adult lymphoid tissue is provided by reciprocal lymphocyte-transfer experiments between mice that are deficient in LT- α (and therefore have no segregation of B and T cells) and wild-type mice⁴⁰. LT- α -deficient lymphocytes segregate normally into B- and T-cell areas after transfer to irradiated wild-type recipients, but the converse does not occur; wild-type lymphocytes that express LT- α are not organized into B- and T-cell areas in LT- α -deficient recipients. As CD4⁺CD3⁻ cells are the main non-lymphoid source of LT- α and LT- β , it seems probable that they are sufficient to provide these signals to stromal cells, which then establish the chemokine gradients that are responsible for the segregation of B and T cells⁴¹.

This model seems to be contradicted, however, by analysis of ROR- γ t-deficient mice³⁴ and inhibitor of DNA binding 2 (ID2)-deficient mice⁴², which lack lymph-node CD4⁺CD3⁻ inducer cells but have almost normal organization of B- and T-cell areas in the spleen. Further analysis of the spleens of these mice should establish whether adult CD4⁺CD3⁻ cells are present. If they are absent, this will indicate that CD4⁺CD3⁻ cells are not essential for the organization of B and T cells in the spleen; however, if they are present, this could indicate that the function of ROR- γ t and ID2 is to allow neonatal CD4⁺CD3⁻ cells to differentiate into a cell type that migrates to the lymph-node anlage and induces lymph-node

formation. In summary, the relationship between adult CD4⁺CD3⁻ cells and neonatal inducer cells is contentious, but we think that they are closely related cell types.

Memory preceded lymph nodes

Assuming that adult CD4⁺CD3⁻ cells and neonatal inducer cells are related, what was the original evolutionary function of cells with this phenotype? Birds and mammals are thought to have evolved from a common reptilian ancestor ~300 million years ago⁴³. In terms of their immune responses, both form GCs when they are immunized, make high-affinity class-switched antibodies, and have B- and T-cell memory. However, birds lack lymph nodes, unlike the most primitive mammals (the monotremes)⁴⁴. This indicates that the genetic and cellular mechanisms for maintaining CD4⁺ memory T cells and therefore for supporting antibody production were established before birds and mammals diverged evolutionarily and before the evolution of lymph nodes.

The key advantage of mammalian immunity is that the main investment that is made during the GC reaction in terms of B-cell-receptor affinity maturation is not lost; the memory response is systemic, not local to the site of initial vaccination, because memory lymphocytes recirculate²¹. After local immune memory was established, animals that were able to redistribute the memory response systemically would have had a selective survival advantage. Lymph nodes provide the infrastructure for a systemic response, and the blood vessels and lymphatics supply the conduit.

Genetic palaeontology supports this proposed evolutionary sequence. The chicken genome (see Chicken Genome Browser in the Online links box) contains orthologues of many genes that are essential for GC development (such as CD28 and CD40), as well as orthologues of CD30 and OX40, which we have proposed are important for T-cell memory. Chickens also have a good orthologue of TNFR1, which has an established role in the segregation of B and T cells⁴⁵, but they lack the master gene for lymph-node development, the gene that encodes LT-βR, which is present in tandem with *TNFR1* in mammalian genomes. We propose that, in the chicken spleen, there is an equivalent of the CD4⁺CD3⁻ cell and that this cell lacks LT-βR but organizes B- and T-cell areas and supports T-cell memory; we also propose that a duplication at the *TNFR1* locus early in mammalian evolution allowed the development of a new LT-βR-dependent function for this cell in lymph-node development (the neonatal inducer cell).

CD4⁺CD3⁻ cells and HIV

Our data link CD4⁺CD3⁻ cells with memory antibody responses and maintenance of the integrity of lymphoid architecture, both of which are lost as HIV infection progresses^{46–48}. CD4⁺CD3⁻ cells in mice express both CD4 and the chemokine receptor CXCR4 (M.-Y.K. and P.J.L.L., unpublished observations), which are co-receptors for virus entry to human cells⁴⁹. Although HIV-positive individuals are infected initially with the CCR5-tropic viral variant, which mainly targets CD4⁺ T-cell populations in the gut⁵⁰, mutation of the virus to the CXCR4-tropic form is associated with the depletion of T cells in secondary lymphoid tissue and with poor prognosis⁴⁹.

High concentrations of HIV are known to be trapped at the surface of follicular DCs in B-cell follicles⁵¹, where CD4⁺CD3⁻ cells could be infected directly. The histopathology of HIV-infected lymph nodes is also consistent with the immunopathology of CD4⁺CD3⁻ cells. Lymph nodes from HIV-infected individuals initially show hyperplastic GC formation; subsequently, the GCs involute, and the follicular architecture disappears⁵². If CD4⁺CD3⁻ cells become targets during the course of HIV infection, not only would this impair the capacity of an individual to produce and maintain neutralizing antibody to the virus and

other pathogens, but also, by destroying the cells that establish and maintain lymphoid architecture, this would undermine immune responses to the numerous, usually non-pathogenic, infections that are associated with AIDS.

How might $CD4^+CD3^-$ cells be destroyed? There are two possibilities: destruction by the virus, or destruction by the host cytolytic $CD8^+$ T-cell response to infected cells. There is some evidence for the latter, because invasion of B-cell follicles by $CD8^+$ T cells is associated with follicular destruction⁵³. In humans, however, the long-term non-progression of untreated HIV infection correlates with strong $CD4^+$ and $CD8^+$ T-cell responses to virus-infected cells. This seems to indicate that $CD4^+CD3^-$ cell destruction by $CD8^+$ T cells is not relevant to AIDS pathogenesis. However, the opposite is true in the natural non-human primate hosts. Chimpanzees⁵⁴ and sooty mangabeys⁵⁵ infected with the simian immunodeficiency virus counterparts of HIV-1 and HIV-2, respectively, show high levels of viraemia without progressive $CD4^+$ T-cell deficiency or AIDS, and this is associated with preservation of the follicular architecture in the former⁵³. This is not because of resistant mutations in the chimpanzee *CXCR4* gene, which is identical to that of humans⁵⁶, but instead there is evidence of a hypoplastic $CD8^+$ T-cell response to the virus^{53,57}, which might allow the survival of a $CD4^+CD3^-$ cell population.

As HIV maintains latency in memory $CD4^+$ T cells and is impossible to eradicate⁵⁸, an ignorant $CD8^+$ T-cell response might have a selective survival advantage in the long term, provided that the virus is not inherently lethal. By preserving $CD4^+CD3^-$ cell function, the probability of raising and maintaining neutralizing antibody responses is increased, which limits the spread of the virus while it preserves immune responses to organisms that are usually non-pathogenic.

Why, then, does this not seem to be the case for human long-term non-progressors? Although there is strong evidence that SIV and HIV mutate to evade recognition by individual MHC molecules⁵⁹, the large number of MHC class I molecules in humans compared with primates might make it difficult for the virus to evade the $CD8^+$ T-cell response entirely. Anything but an all-out assault on virus-infected cells, which seems to be achieved by long-term non-progressors through the strength of their $CD8^+$ T-cell response, allows persistent viraemia and, possibly, the infection of $CD4^+CD3^-$ cells; this leads to the subsequent attrition of the lymphoid architecture that we propose results in AIDS.

Summary

In this article, we have highlighted the importance of $CD4^+CD3^-$ cells in organizing the development and, perhaps, the maintenance of secondary lymphoid organs, as well as in supporting adaptive memory antibody responses. The expression of CD4 and CXCR4 by these cells in mice indicates that $CD4^+CD3^-$ cells might be targets of HIV in humans. Although the immunodeficiency that is associated with HIV is likely to have a multifactorial origin, we think that the destruction of these cells by the host $CD8^+$ T-cell response could account for many of the features that are associated with progressive disease. Pinpointing these cells in humans by identifying the markers that characterize them will help to establish whether these cells are infected and depleted during the course of HIV infection, which will confirm or refute this hypothesis. The result of these experiments is relevant to the control of the HIV pandemic. We predict that, in individuals who make suboptimal responses — that is, those who progress to AIDS — strategies that elicit $CD8^+$ T-cell responses⁶⁰ might aggravate disease, owing to $CD4^+CD3^-$ cell destruction, whereas vaccination strategies that are designed to elicit neutralizing antibody responses⁶¹ are likely to preserve $CD4^+CD3^-$ cells and lymphoid architecture, and improve prognosis.

Acknowledgments

This work was supported by the Wellcome Trust (United Kingdom). We thank I. MacLennan, F. McConnell and G. Anderson for reading the manuscript and providing many helpful comments. We also thank C. Raykundalia, who organized us and made sure that everything in the laboratory worked.

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Figure 1. Location of adult $CD4^+CD3^-CD11c^-B220^-$ cells

a A digitized processed image of an immunostained section from a mouse spleen is shown, indicating the location of $CD4^+CD3^-CD11c^-B220^-$ cells (red) in relation to $CD11c^+$ dendritic cells (DCs; green) and IgM^+ B-cell areas (grey). $CD4^+CD3^-$ cells are mainly located in the region where the T-cell area and the B-cell follicle adjoin (the B–T interface) and adjacent to follicular T cells in B-cell follicles, whereas DCs are located in T-cell areas and the red pulp. Plasma cells are located in the red pulp, which surrounds the white-pulp areas that contain lymphocytes. This image is reproduced with permission from REF. 2 © (2003) Elsevier. **b** A schematic version of this image is shown.

Figure 2. $CD4^+CD3^-$ cells foster the survival of follicular T cells in germinal centres

a In follicles, follicular T (T_F) cells drive the proliferation of B cells, which then differentiate into centroblasts and undergo somatic hypermutation of their immunoglobulin variable gene segments. The centroblasts proliferate rapidly and differentiate into centrocytes, which compete for antigen fragments that are trapped at the surface of follicular dendritic cells (FDCs). B-cell mutants that have high-affinity B-cell receptors internalize antigen and present peptide fragments to T_F cells, which then provide selective CD40 ligand (CD40L)-dependent rescue signals for B cells. When antigen is scarce, T_F cells are sustained by signalling through OX40 and CD30 that is initiated by $CD4^+CD3^-$ cells, which express

OX40 ligand (OX40L) and CD30L constitutively, so that the T_F cells can continue to select B cells. Centrocytes that are positively selected leave the germinal centre (GC) and differentiate into memory B cells or mature into long-lived plasma cells. **b** Before entry to follicles, antigen-specific T cells (white) are primed by dendritic cells (DCs; green) in the T-cell area (white boxes) of lymph nodes. The T cells then upregulate expression of CXC-chemokine receptor 5 (CXCR5) and migrate into B-cell follicles (white arrow). The red box shows an interaction between a T_F cell and a $CD4^+CD3^-$ cell (red) — which allows signalling through OX40 and CD30 in the T_F cell — in a B-cell-follicle GC, and the panel on the left shows a high-magnification view of this association. This image is reproduced with permission from REF. 2 © (2003) Elsevier. BCR, B-cell receptor; Fc receptor, receptor for immunoglobulin; TCR, T-cell receptor.

Figure 3. $CD4^+CD3^-$ cells foster the survival of memory T cells at the B–T interface

a The mechanism for the re-activation of memory T (T_M) cells by $CD4^+CD3^-$ cells is shown. Antigen-specific T_M cells that co-express CXC-chemokine receptor 5 (CXCR5) and CC-chemokine receptor 7 (CCR7) migrate across high endothelial venules (HEVs) into lymph nodes, owing to the expression of ligands for peripheral-node addressin (PNAD) by these cells. Interleukin-7 (IL-7) that is produced possibly by stromal cells or follicular dendritic cells (FDCs) in lymphoid tissue upregulates the expression of OX40 at the surface of T_M cells. These cells then interact with $CD4^+CD3^-$ cells, which express OX40 ligand (OX40L) constitutively, in the region where the T-cell area and the B-cell follicle adjoin (the B–T interface). The interaction of $CD4^+CD3^-$ cells with T_M cells provides survival signals to T_M cells through OX40, by inducing the upregulation of B-cell lymphoma 2 (BCL-2) and BCL- X_L expression. **b** An image of the B–T interface is shown. The inset shows a high-magnification view of the interaction of a $CD4^+CD3^-$ cell (red) with a T_M cell (white). This image is reproduced with permission from REF. 2 © (2003) Elsevier.