Role of B cells in maintaining helper T−cell memory

Dominic van Essen, Per Dullforce and David Gray

References	Article cited in: **http://rstb.royalsocietypublishing.org/content/355/1395/351#related-urls**
Email alerting service	Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

THE ROYAL SOCIETY

Role of B cells in maintaining helper T-cell memory

Dominic van Essen†, Per Dullforce‡ and David Gray*

Institute of Cell, Animal and Population Biology, University of Edinburgh, Ashworth Laboratories, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK

The cellular interactions involved in maintaining CD4$^+$ T-cell memory have hitherto not been identified. In this report, we have investigated the role played by B cells in this process. We show that that long-lasting helper T-cell memory depends on the presence of B cells, but that direct antigen presentation by B cells is not required. These findings provide new insights into the mechanisms which underlie helper T-cell memory. They also suggest that the efficacy of future vaccines will depend critically on the inclusion of B- as well as T-cell epitopes.

Keywords: B cells; CD4$^+$ T cells; antibody; antigen presentation; follicular dendritic cells; antigen dependence

1. INTRODUCTION

Immunological T-cell memory is an area of intense interest (for reviews, see Ahmed & Gray 1996; Sprent 1994), but a consensus has yet to emerge as to how it is generated and maintained. Rival theories state either that memory T cells are specialized, long-lived clones (Bruno *et al*. 1995), or that they comprise a population needing continual stimulation by antigen in order to persist (Gray & Matzinger 1991).

In the case of CD8$^+$ T-cell memory, recent reports suggest that persisting antigen may not be required (Mullbacher 1994; Lau *et al*. 1994; Hou *et. al*. 1994): instead, cross-reactivity with other antigens (Selin *et al*. 1994; Beverley 1990) and activation by cytokines (Tough *et al*. 1996; Zhang *et al*. 1998) have both been suggested as means by which CD8$^+$ memory T cells are maintained. In contrast, few studies have addressed the requirements for maintaining CD4$^+$ T-cell memory (Gray & Matzinger 1991). Bearing in mind the differences in the nature of antigens recognized by CD8$^+$ and CD4$^+$ T cells (intracellular versus extracellular), and in the cell types which can present them (almost all cells express major histocompatibility (MHC) class I, whereas only specialized antigen-presenting cells (APCs) express MHC class II), it seems likely that CD8$^+$ and CD4$^+$ T-cell memory could be maintained by different mechanisms.

It has long been suggested (Feldbush 1973) that intermittent stimulation by persisting antigen is necessary for the survival of both memory helper T cells (Gray & Matzinger 1991) and memory B cells (Gray & Skarvall 1988). The only known means by which non-replicating antigens can be retained *in vivo* for long periods is in the form of immune complexes on the surface of follicular dendritic cells (FDCs; Mandel *et al*. 1980). If these are required to maintain helper T-cell memory, it follows that this memory should depend on the production of antibodies by B cells. We tested this directly by examining the longevity of CD4$^+$ T-cell memory in mice which lack B cells.

2. MATERIAL AND METHODS

(a) *Animals*

B6 µMT (Kitamura & Rajewsky 1992), Bl0 severe combined immunodeficient (SCID), C57BL/6 and Bl0.D2/n mice were six to ten weeks old at the start of each experiment. Mice were maintained on sterile food and bedding in filter-topped cages. Thymi were taken from C57BL/6 foetuses at day 14 of gestation and up to six were implanted under the kidney capsules of Bl0 SCID mice. Six to ten weeks later, reconstitution of T cells in peripheral blood leucocytes (PBL) was confirmed by fluorescence-activated cell sorter (FACS) analysis using fluorescein isothiocyanate (FITC)-labelled anti-Thy-1 (provided by H. von Boemer). B6 µMT mice were backcrossed with congenic Bl0.D2/n mice, and expression of H-2K^b and H-2D^d on PBLs was assessed by FACS analysis using biotinylated monoclonal antibodies AF6–88 and 19–191 (both provided by A. Livingstone), respectively, followed by PE-streptavidin. B220 expression was detected using FITC-labelled RA3–6B2 (Pharmingen, San Diego, CA, USA).

(b) *B-cell reconstitution and priming*

Bl0 SCID mice and B6 µMT mice were reconstituted intravenously with 10^7 B cells purified (Kapasi *et al*. 1993) from non-immune C57BL/6 mice. These mice were immunized intraperitoneally at the same time with 50 µg keyhole limpet haemocyanin (KLH) (Calbiochem, San Diego, CA, USA). KLH, possibly due to its large size, is one of the few antigens which is immunogenic in this form. To prevent possible attack by natural killer-cells, H-2$^{b/d}$ µMT mice were irradiated with 5 Gy one day before the injection of 10^7 B cells from C57BL/6 or

351

Bl0.D2/n mice. These mice were immunized one week later, to allow time for the regeneration of T cells. Two weeks after immunization, all mice were bled and serum immunoglobulin levels and anti-KLH IgG and IgM were measured as described (Gray *et al.* 1994). At the longest time-point analysed (12 weeks), donor B cells were still detectable in splenocytes by FACS analysis of B220 expression (as above).

(c) *Limiting dilution analysis*

Frequencies of KLH-specific IL-2 producing T cells were measured in reconstituted SCID and H-2^b μMT mice as described (Van Essen *et al.* 1995). All LDAs had χ^2-test probabilities of >0.05, indicating that the data was well fitted by a Poisson distribution of responder cells. To measure H-2^b- and H-2^d-restricted T-cell frequencies in reconstituted H-2$^{b/d}$ μMT mice, CD4$^+$ T cells were highly purified from responder splenocytes by depletion of MHC class II$^+$, CD8$^+$, CD11c$^+$ and IgM$^+$ cells using biotinylated monoclonal antibodies M5/114 (ATCC), 53.6.72 (ATCC) and N418 (32), and goat anti-mouse IgM (Southern Biotechnology Associates, Birmingham, UK), respectively, followed by two rounds of MACS separation (Miltenyi Biotec, Bergisch Gladbach, Germany). Purity of sorted cells was routinely $>99.5\%$, and these cells did not proliferate in response to either KLH or immobilized anti-CD$_3$ (145.2Cl1) unless exogenous APCs were added. Otherwise, limiting dilution analysis was as above. It should be noted that T cells restricted by hybrid H-2^b/H-2^d MHC molecules (e.g. I-E^b) will not be detected in these analyses.

(d) *Analysis of MHC restriction of antigen-specific T cells*

2×10^6 purified CD4$^+$ T cells, from an H-2$^{b/d}$ μMT mouse given H-2^d (C57BL/6) B cells, were cultured with an equal number of irradiated (30 Gy) H-2^d (Bl0.D2/n) APCs plus $100 \mu g \, ml^{-1}$ KLH and $100 \, U \, ml^{-1}$ IL-2. Two weeks later, cells were washed and re-cultured at 100 cells per well with 2×10^5 APCs (either H-2^b or H-2^d) and $100 \, U \, ml^{-1}$ IL-2, with and without KLH. After a further two weeks, wells were scored microscopically for cell growth. Numbers of positive wells per number of total wells: H-2^b APC + KLH, 0/96; H-2^b APC alone, 0/96; H-2^d APC + KLH, 16/96; H-2^d APC alone, 0/96 ($p < 10^{-4}$, Fisher's exact test).

3. RESULTS AND DISCUSSION

SCID mice have neither T nor B cells, but T cells can be regenerated in these mice by implantation of foetal thymi from wild-type mice. We immunized mice treated in this way with soluble KLH, and injected half of them at the same time with syngeneic B cells. Soluble antigen was used in order to minimize the possibility of forming any artificial deposits *in vivo*—thus, retention of antigen should occur solely by the trapping of immune complexes on FDCs. Two weeks after immunization, all the mice which had received B cells had produced serum anti-KLH IgM and IgG antibodies, indicating successful T- and B-cell priming (not shown). Ten weeks after immunization, we measured the frequencies of KLH-specific helper T cells in each mouse. At this stage, the primary immune response has long since finished, and the continued presence of elevated numbers of antigen-specific cells is indicative of immunological memory. We found that those mice which had received B cells had significantly higher numbers of
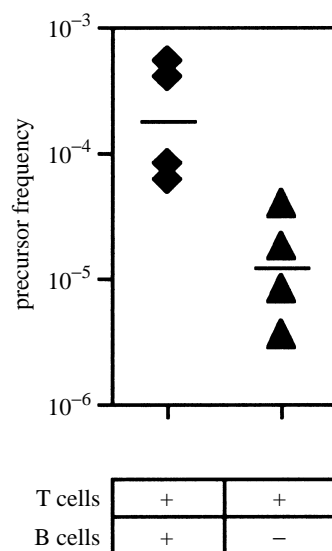

Figure 1. CD4$^+$ T-cell memory is impaired in T-cell-reconstituted SCID mice without B cells. Frequencies of KLH-specific helper T cells were assayed by limiting dilution analysis of IL-2 producing cells, ten weeks after immunization with $50 \mu g$ soluble KLH. SCID mice were grafted with thymi from syngeneic C57BL/6 mice, and half received 10^7 C57BL/6 B cells intravenously at the same time as immunization (diamonds). The remainder did not receive any B cells (triangles). Each point represents the frequency in one mouse. Geometric mean frequencies (lines): SCID + T cells and B cells, 1.8×10^{-4}, SCID + T cells only, 1.2×10^{-5} ($p = 0.012$, two-tailed, unpaired Student's t-test). Unimmunized mice typically exhibit frequencies of less than 2×10^{-6} (not shown).

KLH-specific helper T cells than those which had not (figure 1). This demonstrates that CD4$^+$ T-cell memory is impaired in the absence of B cells.

There are two alternative explanations for the above result: B cells could be needed (i) for the maintenance of helper T-cell memory, or (ii) for the initial clonal expansion of antigen-specific T cells. We investigated the latter possibility by measuring the frequencies of antigen-specific T cells at the peak of the primary response, two weeks after immunization. The experimental protocol was essentially as above, except that μMT mice were used in place of SCID mice. μMT mice have a disrupted immunoglobulin $C\mu$ gene, resulting in the complete absence of mature B cells in the periphery (Kitamura & Rajewsky 1992). Again, mice were immunized with soluble KLH, and half were injected with syngeneic B cells. Two weeks later, only those mice which had received B cells had produced serum anti-KLH antibodies (not shown). We measured the frequencies of KLH-specific helper T cells after two weeks, to assess their initial clonal expansion, and again after 12 weeks, to test for T-cell memory. As shown in figure 2*a*, two weeks after immunization there was no difference in the level of T-cell priming between mice which had, and which did not have, B cells. This is in line with another recent report on T-cell priming in B-cell-deficient mice (Epstein *et al.* 1995). After 12 weeks, as with the SCID mice, the number of antigen-specific T cells has decayed more rapidly in μMT mice which have not been given B cells (figure 2*b*). These data confirm that while B cells are not needed for the initial expansion of antigen-specific T cells,
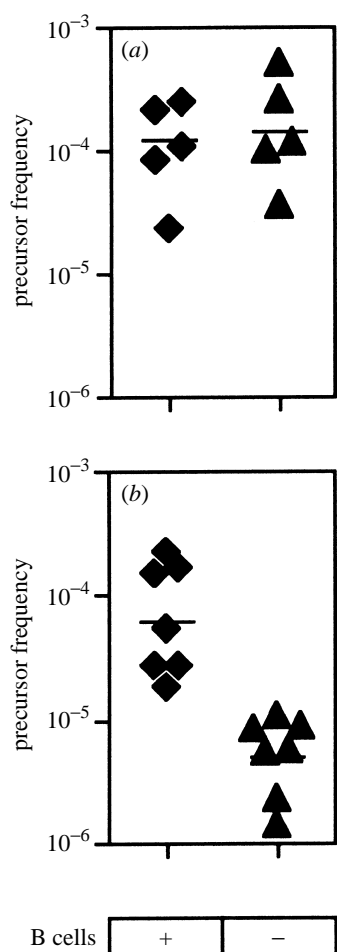
Figure 2. CD4^{+} T-cell memory, but not priming, is impaired in μMT mice without B cells. Frequencies of KLH-specific helper T cells in μMT mice, (a) two weeks, and (b) 12 weeks after immunization with KLH. Mice were immunized and given C57BL/6 B cells as in figure 1. Geometric mean frequencies: (a) μMTs + B cells (diamonds) 1.4×10^{-4}, μMTs without B cells (triangles), 1.3×10^{-4} ($p = 0.81$); (b) μMTs + B cells, 6.3×10^{-5}, μMTs without B cells, 5.1×10^{-6} ($p = 2.6 \times 10^{-4}$).

they are vital for the maintenance of helper T-cell memory.

Topham *et al.* (1996) have previously studied CD4^{+} T-cell memory to influenza virus infection in μMT and wild-type mice. In contrast to the results presented above, they concluded that B cells were not required for long-lasting helper T-cell memory. However, the use of live virus as an immunogen makes it difficult to exclude the possibility of incomplete viral clearance, thereby bypassing any need for antigen localization on FDCs. This is particularly relevant in immunocompromised animals, and indeed several reports suggest that B cells are important for the elimination of influenza viruses (Iwasaki & Nozima 1977; Kris *et al.* 1988; Palladino *et al.* 1995). Although the authors found it to be effectively cleared from the site of infection (lungs), this virus is able to infect all cell types, as its receptor, sialic acid, is ubiquitously expressed.

While FDCs can bind and retain immune complexes on their surface for long periods of time, they are unable to endocytose and process them for presentation to T cells

(Gray *et al.* 1991). For this, an intermediary APC is needed. Previous studies have shown that B cells are able to acquire antigen by the endocytosis of bead-like, immune-complex-coated bodies (iccosomes), derived from the dendrites of FDCs (Szakal *et al.* 1988), and that they can present this antigen to CD4^{+} T cells *in vitro* (Gray *et al.* 1991; Kosco *et al.* 1988). We therefore investigated the requirement for antigen presentation by B cells to maintain helper T-cell memory. Since mice which lack B cells not only cannot form immune complexes, but also cannot support the development of FDCs (MacLennan & Gray 1986; Kapasi *et al.* 1993), we generated chimaeric mice which contained B cells, but in which half of the T cells could not recognize the antigen presented by them. We backcrossed μMT mice (H-2[b]) onto the H-2[d] genetic background, and generated F$_1$ mice expressing both *b* and *d* alleles at the H-2 locus (H-2[b/d] μMT mice). These mice contain T cells which recognize antigen presented on either H-2[b] or H-2[d] MHC molecules. We then reconstituted these mice with H-2[b] B cells: the resulting chimaeric animals generated antibody responses when immunized (not shown), but only H-2[b]-restricted T cells can recognize antigen presented by B cells. We reconstituted a second group of mice in the reverse fashion, using H-2[d] B cells. All mice were then immunized as before, with soluble KLH.

Two weeks and 12 weeks later, we performed limiting dilution analyses of the CD4^{+} T cell from these mice, using either H-2[b]- or H-2[d]-expressing APCs. In this way, we were able to independently determine the frequencies of H-2[b]- and H-2[d]-restricted KLH-specific T cells. Figure 3a,b illustrates that two weeks after immunization, there was no difference in the level of T-cell priming between these two populations. This corroborates our earlier finding that clonal expansion proceeds normally in the absence of B cells. Twelve weeks after immunization, we found, to our surprise, that antigen-specific T cells which could not recognize antigen presented by B cells were still present at elevated levels, which were comparable to the levels of antigen-specific T cells that could do so (figure 3c,d). Thus, it seems that while B cells are required for the survival of CD4^{+} memory T-cells (figures 1 and 2), they are not needed as APCs (figure 3). It remained possible that the same T cell could recognize KLH-derived peptides presented on both H-2[b] and H-2[d] MHC molecules. This is unlikely, in view of the differences in structure and in peptide-binding preferences (Brusic *et al.* 1997) between I-A[b] and I-A[d] molecules. Nevertheless, we verified that this did not occur by stimulating bulk cultures of CD4^{+} T cells from an H-2[b/d] μMT mouse given H-2[d] B cells, using H-2[d]-expressing APCs. After two weeks, we purified and re-stimulated these T cells under limiting dilution conditions using either H-2[d]- or H-2[b]-expressing APCs. Whereas clones of T cells were readily stimulated by APCs expressing H-2[d], none were stimulated by APCs expressing H-2[b]. This confirms that H-2[d]-restricted T cells in this mouse could not recognize antigen presented on H-2[b] MHC molecules, thereby strengthening the notion that CD4^{+} T-cell memory does not depend on antigen presentation by B cells.

How, then, does the presence of B cells affect the persistence of helper T-cell memory? The data outlined here support a model in which antibody production by B cells is needed for generation of immune complexes,
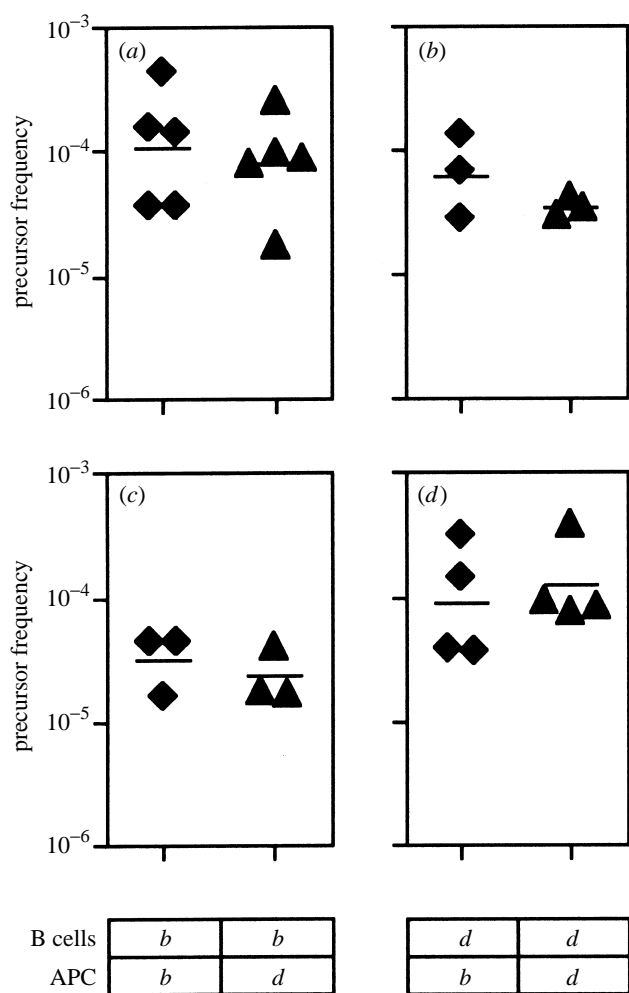
Figure 3. CD4^{+} T-cell memory does not require antigen presentation by B cells. Frequencies of H-2[b]- and H-2[d]-restricted KLH-specific helper T cells in H-2[b/d] μMT mice at (a,b) two weeks and (c,d) 12 weeks after immunization with KLH. H-2[b/d] μMT mice were lightly irradiated and reconstituted with 10^7 B cells from congenic H-2[b] (C57BL/6 (a,c)), or H-2[d] (B10.D2/n (b,d)) donors. One week later they were immunized as in figure 1. Geometric mean frequencies: (a) H-2[b]-restricted (diamonds), 1.1×10^{-4}, H-2[d]-restricted (triangles), 7.9×10^{-5} ($p = 0.41$, two-tailed, paired Student's t-test); (b) H-2[b]-restricted, 6.2×10^{-5}, H-2[d]-restricted, 3.4×10^{-5} ($p = 0.40$); (c) H-2[b]-restricted, 3.2×10^{-5}, H-2[d]-restricted, 2.3×10^{-5} ($p = 0.53$); (d) H-2[b]-restricted, 9.1×10^{-5}, H-2[d]-restricted, 1.2×10^{-4} ($p = 0.66$).

and thus for long-term retention of antigen. This antigen is periodically presented to memory CD4^{+} T cells, providing a stimulus for their survival, by APCs which are not B cells. This theory demands that there are cell types other than B cells which can acquire antigen from FDCs and present it to T cells. One promising candidate for this is the recently described germinal centre dendritic cell (Grouard et al. 1996). These cells express complement and F_c receptors and can bind immune complexes, but unlike interdigitating dendritic cells they have minimal ability to take up soluble antigens. *In vivo* they are found in germinal centres, in close proximity both to T cells and to FDCs. Experiments are underway to investigate whether these cells are involved in the maintenance of

T-cell memory. The results do not rule out the possibility that B cells provide a trophic signal (cytokine?) that enhances CD4^{+} memory T-cell survival in a manner similar to the influence of type I interferons and IL-15 on CD8^{+} memory cells (Tough et al. 1996; Zhang et al. 1998). However, recent results (D. van Essen, A. Cutler and D. Gray, unpublished data) have shown that Clq-deficient mice (Cutler et al. 1998), which do not localize antigens to FDC, also have impaired maintenance of CD4^{+} T-cell memory.

In conclusion, we have shown that CD4^{+} T-cell memory decays in the absence of B cells. This is markedly different from the published reports on CD8^{+} T-cell memory (Asano & Ahmed 1996; Di Rosa & Matzinger 1996), implying that CD4^{+} and CD8^{+} memory T cells are not maintained by the same mechanism. Moreover, we have demonstrated that the role of B cells in prolonging helper T-cell memory is not as APCs. This poses an important new question: What cell type(s) are required to present antigen to CD4^{+} memory T cells?

REFERENCES

Ahmed, R. & Gray, D. 1996 Immunological memory and protective immunity: understanding their relation. *Science* **272**, 54–60.

Asano, M. S. & Ahmed, R. 1996 CD8 T cell memory in B cell-deficient mice. *J. Exp. Med.* **183**, 2165–2174.

Beverley, P. C. 1990 Is T-cell memory maintained by cross-reactive stimulation? *Immunol. Today* **11**, 203–205.

Bruno, L., Kirberg, J. & Von Boehmer, H. 1995 On the cellular basis of immunological T cell memory. *Immunity* **2**, 37–43.

Brusic, V., Rudy, G., Kyne, A. P. & Harrison, L. C. 1997 MHCPEP, a database of MHC-binding peptides: update 1997. *Nucl. Acids Res.* **25**, 269–271.

Cutler, A., Botto, M., Van Essen, D., Rivi, R., Davies, K., Gray, D. & Walport, M. 1998 T-dependent immune response in Clq-deficient mice: defective IFN-γ production by antigen-specific T cells. *J. Exp. Med.* **187**, 1789–1797.

Di Rosa, F. & Matzinger, P. 1996 Long-lasting CD8 T cell memory in the absence of CD4 T cells or B cells. *J. Exp. Med.* **183**, 2153–2163.

Epstein, M. M., Di Rosa, F., Jankovic, D., Sher, A. & Matzinger, P. 1995 Successful T cell priming in B cell-deficient mice. *J. Exp. Med.* **182**, 915–922.

Feldbush, T. L. 1973 Antigen modulation of the immune response. The decline of immunological memory in the absence of continuing antigenic stimulation. *Cell. Immunol.* **8**, 435–444.

Gray, D. & Matzinger, P. 1991 T cell memory is short-lived in the absence of antigen. *J. Exp. Med.* **174**, 969–974.

Gray, D. & Skarvall, H. 1988 B-cell memory is short-lived in the absence of antigen. *Nature* **336**, 70–73.

Gray, D., Kosco, M. & Stockinger, B. 1991 Novel pathways of antigen presentation for the maintenance of memory. *Int. Immunol.* **3**, 141–148.

Gray, D., Dullforce, P. & Jainandunsing, S. 1994 Memory B cell development but not germinal center formation is impaired by in vivo blockade of CD40–CD40 ligand interaction. *J. Exp. Med.* **180**, 141–155.

Grouard, G., Durand, I., Filgueira, L., Banchereau, J. & Liu, Y. J. 1996 Dendritic cells capable of stimulating T cells in germinal centres. *Nature* **384**, 364–367.

Hou, S., Hyland, L., Ryan, K. W., Portner, A. & Doherty, P. C. 1994 Virus-specific CD8$^+$ T-cell memory determined by clonal burst size. *Nature* **369**, 652–654.

Iwasaki, T. & Nozima, T. 1977 Defense mechanisms against primary influenza virus infection in mice. I. The roles of interferon and neutralizing antibodies and thymus dependence of interferon and antibody production. *J. Immunol.* **118**, 256–263.

Kapasi, Z. F., Burton, G. F., Shultz, L. D., Tew, J. G. & Szakal, A. K. 1993 Induction of functional follicular dendritic cell development in severe combined immunodeficiency mice. Influence of B and T cells. *J. Immunol.* **150**, 2648–2658.

Kitamura, D. & Rajewsky, K. 1992 Targeted disruption of mu chain membrane exon causes loss of heavy-chain allelic exclusion. *Nature* **356**, 154–156.

Kosco, M. H., Szakal, A. K. & Tew, J. G. 1988 *In vivo* obtained antigen presented by germinal center B cells to T cells *in vitro*. *J. Immunol.* **140**, 354–360.

Kris, R. M., Yetter, R. A., Cogliano, R., Ramphal, R. & Small, P. A. 1988 Passive serum antibody causes temporary recovery from influenza virus infection of the nose, trachea and lung of nude mice. *Immunology* **63**, 349–353.

Lau, L. L., Jamieson, B. D., Somasundaram, T. & Ahmed, R. 1994 Cytotoxic T-cell memory without antigen. *Nature* **369**, 648–652.

MacLennan, I. C. & Gray, D. 1986 Antigen-driven selection of virgin and memory B cells. *Immunol. Rev.* **91**, 61–85.

Mandel, T. E., Phipps, R. P., Abbot, A. & Tew, J. G. 1980 The follicular dendritic cell: long term antigen retention during immunity. *Immunol. Rev.* **53**, 29–59.

Metlay, J. P., Witmer-Pack, M. D., Agger, R., Crowley, M. T., Lawless, D. & Steinman, R. M. 1990 The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. *J. Exp. Med.* **171**, 1753–1771.

Mullbacher, A. 1994 The long-term maintenance of cytotoxic T cell memory does not require persistence of antigen. *J. Exp. Med.* **179**, 317–321.

Palladino, G., Mozdzanowska, K., Washko, G. & Gerhard, W. 1995 Virus-neutralizing antibodies of immunoglobulin G (IgG) but not of IgM or IgA isotypes can cure influenza virus pneumonia in SCID mice. *J. Virol.* **69**, 2075–2081.

Selin, L. K., Nahill, S. R. & Welsh, R. M. 1994 Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. *J. Exp. Med.* **179**, 1933–1943.

Sprent, J. 1994 T and B memory cells. *Cell* **76**, 315–322.

Szakal, A. K., Kosco, M. H. & Tew, J. G. 1988 A novel *in vivo* follicular dendritic cell-dependent iccosome-mediated mechanism for delivery of antigen to antigen-processing cells. *J. Immunol.* **140**, 341–353.

Topham, D. J., Tripp, R. A., Hamilton Easton, A. M., Sarawar, S. R. & Doherty, P. C. 1996 Quantitative analysis of the influenza virus-specific CD4$^+$ T cell memory in the absence of B cells and Ig. *J. Immunol.* **157**, 2947–2952.

Tough, D. F., Borrow, P. & Sprent, J. 1996 Induction of bystander T cell proliferation by viruses and type I interferon *in vivo*. *Science* **272**, 1947–1950.

Van Essen, D., Kikutani, H. & Gray, D. 1995 CD40 ligand-transduced co-stimulation of T cells in the development of helper function. *Nature* **378**, 620–623.

Zhang, X., Sun, S., Hwang, I., Tough, D. & Sprent, J. 1998 Potent and selective stimulation of memory-phenotype CD8$^+$ T cells *in vivo* by IL-15. *Immunity* **8**, 591–599.