CD45RC Isoforms Define Two Types of CD4 Memory T Cells, One of which Depends on Persisting Antigen

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Summary

The cellular basis of immunological memory remains a controversial area with respect to the identity of memory T cells and the role of persisting antigen. CD4 T cells are phenotypically divided by the expression of high and low molecular weight isoforms of CD45, surface markers that are frequently used to identify "naive" (CD45R\textsuperscript{high}) and "memory" (CD45R\textsuperscript{low}) subsets. The latter subset responds rapidly in antigen recall assays but paradoxically has a short life span, a property that is difficult to reconcile with long-term memory. The present study examines these issues using a DTH (delayed-type hypersensitivity) model in which contact sensitivity to dinitrochlorobenzene (DNCB) was transferred to athymic nude rats by recirculating CD4 T cell subsets defined in the rat by the anti-CD45RC mAb OX22. As expected, CD45RC\textsuperscript{1} (but not RC\textsuperscript{2}) CD4 T cells from normal unprimed rats transferred a DNCB-specific DTH response, whereas, 4 d after sensitization the CD45RC\textsuperscript{2} (memory) subset alone contained the DNCB reactivity. However, when donor cells were collected from thymectomized rats sensitized two mo earlier, DNCB-specific responses were transferred by both CD45RC\textsuperscript{2} and RC\textsuperscript{1} subsets suggesting that many of the latter had developed from cells with a memory phenotype. This was confirmed when CD45RC\textsuperscript{2} CD4 T cells from 4-d primed rats were parked in intermediate nude recipients and recovered 2 mo later. DNCB-specific activity was now found wholly within the CD45RC\textsuperscript{1} "revertant" subset; the CD45RC\textsuperscript{2} CD4 T cell population was devoid of activity. Importantly, we found that the total switch-back from CD45RC\textsuperscript{2} to RC\textsuperscript{1} could be prevented, apparently by persisting antigen. The results indicate that there are two functionally distinct categories of memory T cells: one, a short-lived CD45R\textsuperscript{low} type which orchestrates the rapid kinetics, the other, a longer-lived CD45R\textsuperscript{high} revertant which ensures that immunological memory endures.

A hallmark of the immune response is memory, the ability to recall an earlier encounter with specific antigen, often years later, by producing a faster and larger response. A definitive investigation by Gowans and Uhr (1) using adoptive transfer, squarely laid the basis of immunological memory with recirculating lymphocytes recovered from the thoracic duct. At the time, it was generally accepted that immunological memory was vested in lymphocytes, which, after an initial antigen encounter, were imbued with the quality of long life (2). This view was challenged by the experiments of Gray and colleagues (3, 4) that suggested that the survival of both B and T memory cells depended critically on the persistence of antigen. The role that persisting antigen plays in maintaining memory has been reexamined recently for CD8 T cells specific for viruses (5–9). The balance of evidence suggests that in the absence of antigen, specific CD8 T cells may survive, although some argue that protective immunity by CD8 T cells ultimately depends on the persistence of antigen (8, 9). To what extent CD4 T cell memory is governed by residual antigen has not been resolved.

T cells stimulated by antigen undergo a number of well-described changes. The question is which of these, if any, are retained and can be used to identify memory T cells. The increased expression of CD44 after antigen stimulation has been used to identify CD8 and CD4 memory T cells in mice (10). In some mouse strains, but not others (11), CD44 remains high on CD8 T cells for many months (9, 11, 12), but the high density is not maintained on CD4 T cells (12). The idea that memory T cells exclusively recirculate to lymph nodes via afferent lymphatics (13, 14) is not supported by later studies that show that cells with a memory phenotype readily cross high endothelial venules within lymph nodes (15, 16). The downregulation of L-selectin (CD62L) (9, 17–19) and the increased expression of the \(\alpha_4\) integrin subunit (CD49d) (9, 19) that alters migration have also been used as markers of memory T cells, but modulation of these adhesion molecules is reversible and closely linked with the...
activation state of the cell (9, 12, 18). The high and low molecular weight isomers of CD45R are consistently used to identify naïve and memory CD4 (but not CD8 [18]) T cells (20–22). CD45R<sup>low</sup> CD4 T cells have rapid in vitro antigen recall responses (23, 24) and are able to help primed B cells produce antibody (21, 22), functions which suggest memory T cells. Equating the CD45R<sup>low</sup> phenotype with memory, however, poses a dilemma. Whereas CD45R high (naïve) T cells are nondividing long-lived cells, the CD45R<sup>low</sup> subset tends to be a rapidly dividing, short-lived population (12, 27–29). Furthermore, CD45R<sup>low</sup> CD4 T cells upregulate Fas (CD95) and downregulate bcl-2 (30, 31), a phenotype associated with impending apoptosis. It is difficult to reconcile a short-lived cell with the fact that immunological memory endures.

The antigen-driven switch from CD45R<sup>high</sup> to CD45R<sup>low</sup> (32, 33) was initially considered to be one-way (20, 23, 24). In vivo studies in rats (34) and subsequently in humans (27) indicated that CD45R<sup>low</sup> isoform expression was reversible. Although the time-frame for humans may be longer (29), the speed with which rat CD4 T cells could revert in vivo from CD45R<sup>low</sup> to CD45R<sup>high</sup> was striking (28); a majority of CD4 T cells had switched back to the CD45R<sup>high</sup> phenotype within a week. This implied that T cells that have previously encountered antigen, i.e., memory T cells, should be found in both CD45R<sup>low</sup> and CD45R<sup>high</sup> subsets. Direct evidence for this supposition is lacking. It has also been suggested that persisting antigen could control CD45R isoform expression (35), but this issue has not yet been addressed for CD4 T cells.

In the present investigation, we used a delayed-type hypersensitivity (DTH)<sup>1</sup> model in which a specific response to the contact sensitizing agent 2,4-dinitrochlorobenzene (DNCB) could be adoptively transferred to athymic nude rats by recirculating CD4 T cells. By using allototype-marked donor cells we were able to follow changes in CD45R isoform expression after transfer. The results demonstrated that antigen-experienced CD4 memory T cells revert en masse (default) from CD45R<sup>low</sup> to CD45R<sup>high</sup>. Importantly though, residual antigen could prevent the total switchback from occurring.

### Materials and Methods

Rats. Congenic allototype-marked PVG-R T<sup>1</sup>-R T<sup>7</sup> and athymic PVG-R T<sup>1</sup>-nu/nu(R T<sup>7</sup>) nude rats were bred and maintained under conventional conditions in the Animal Unit, University of Manchester Medical School, and used at 2–3 mo of age. In some experiments PVG-R T<sup>1</sup>-R T<sup>7</sup> rats were thymectomized at 4–6 wk of age and rested for 1 mo before use.

Induction and measurement of contact sensitivity. Rats were sensitized by applying 200 μl of a 5% solution of DNCB (BDH Chemicals Ltd., Poole, UK) in ethanol on the shaved abdomen. On day 4 after sensitization rats were challenged by applying to both sides of the ear a total of 40 μl of a 1% solution of DNCB (400 μg) in ethanol to the left ear and 40 μl of ethanol only to the right ear. Ear thickness was measured 24 h after challenge (time of maximum response) using a spring-loaded engineer’s micrometer (ODITEST ODI OOT; Kröplin GMBH, Hessen, Germany) by full release of the spring. The response was then recorded as the difference in ear thickness between the left and right ears and expressed as μm ± SD. Differences between groups were compared using Student’s t-test.

Antibodies. The following mAbs were used for cell separation and phenotyping: OX 6 (anti-class II MHC), OX 7 (anti-Thy-1), OX 8 (anti-CD8), OX 12 (anti-Igk chain), OX 22 (anti-CD45R C), OX 40 (against a molecule with some homology to CD40 and nerve growth factor receptor; present on activated CD4 T cells [36]), W 3/25 (anti-CD4), R73 (anti-αβ TCR) and 8G6.1 (rat anti-R T<sup>7</sup>; a gift from Dr. Simon Hunt, Oxford). Mouse mAbs were detected with FITC-conjugated F(ab’)<sub>2</sub>, rabbit anti-mouse Ig (Dako Ltd., High Wycombe, UK) absorbed extensively against rat Ig. Biotinylated mAbs were detected with phycoerythrin-streptavidin (Sera-lab, Cowley, UK). Mouse mAbs were purchased from Serotec (Bicester, Oxon, UK) or produced as ascites in the laboratory by injecting the relevant hybridoma into pristine-primed Balb/c mice. The R73 clone (37) was a gift from Prof. T. Hünig (Würzburg, Germany). Other hybridoma cell lines were provided by ECAAC (Porton Down, UK). The dual fluorescence staining technique used to phenotype allototype-marked (R T<sup>7</sup>) donor cells after transfer to wild-type (R T<sup>7</sup>) recipients was described elsewhere (28).

Cell Separation. Lymphocytes from lymph nodes (LN) or the thoracic duct (TDL) were incubated for 30 min at 4°C with saturating concentrations of OX 6, OX 8, OX 12, and OX 7 (to remove B cells, CD8<sup>+</sup> T cells and CD45R<sup>−</sup> T cells) plus 2% fetal calf serum and mixed on a roller/rocker at 4°C. The remaining CD4 T cells (<98% CD4<sup>+</sup>) were incubated with saturating concentrations of mAb OX 22 (anti-CD45R C), positively selected onto goat anti-mouse IgG-coated Biomag<sup>TM</sup> particles (Dynatech Diagnostics Ltd., Piddington, UK). The resulting rosettes were removed from suspension by a magnetic (Dynal, Ltd., New Ferry, UK) applied to the wall of the container. The remaining CD4 T cells (<98% CD4<sup>+</sup>; FITC-conjugated W 3/25) were incubated with saturating concentrations of mAb OX 22 (anti-CD45R C), positively selected onto goat anti-mouse-coated Biomag<sup>TM</sup> particles, the cell/particle rosettes washed free of unbound CD45R<sup>−</sup> C T cells, resuspended at appropriate concentrations in the CD45R<sup>−</sup> C T cells injected intravenously with particles attached. The unbound nonrosetting cells were retained with OX 22, mixed with Biomag<sup>TM</sup> particles and depleted of mAb-stained cells as above by 2 or 3 rounds of magnetic adhesion. The CD45R<sup>−</sup> CD4 T cells (<98% pure) were injected intravenously.

### Results

Kinetics of response in euthymic rats. The DNCB-induced DTH response, recorded as the difference between left (DNCB in ethanol) and right ears (ethanol only control), was always recorded 24 h after ear painting, i.e., challenge.
To determine the kinetics of the response, rats were sensitized, i.e., DNCB applied to the abdomen, and ear-challenged on days 1-18. The maximum response to challenge (410 μm ± 82) was induced 4 d after primary sensitization and declined if challenge was delayed further. The response to DNCB subsided rapidly to a plateau of around 120 μm by day 8, a typical response pattern reported by others (39).

Adoptive transfer to nude recipients. A series of experiments showed that the DNCB response could be adoptively transferred to athymic PVG-RT1u nude rats by whole LN cells or by CD4 T cells purified from TDL of unprimed PVG-RT1u rats. To standardize the procedure, we opted to transfer 10^6 T cells 3 wk before sensitization, an interval designed to allow donor T cells to spontaneously proliferate in nude recipients (40). Ears were always challenged 4 d after sensitization and ear thickness measured 24 h later. The assay was designed to identify the presence or absence of DNCB-specific T cells in the cell inoculum and, within limits, the size of the response was cell dose dependent (data not shown).

In the rat CD4 T cells are divided into two functionally distinct subsets by the anti-CD45R mAb OX22 (25, 26) that detects an epitope on the C exon product of CD45R (41). Exon C is one of three (A, B, or C) that can be variably spliced out of the CD45R molecule by posttranslational mRNA transcription (42, 43) and is expressed (CD45R C+) by three-quarters of the rat CD4 T cell population (25, 34). T cells emerge from the thymus (recent thymic emigrants, RTE) with the phenotype CD45RC- Thy-1+ and develop within a week into mature CD45RC+ Thy-1- T cells (38, 44). Thus, CD45RC- CD4 T cells in the periphery are a mixture of immature Thy-1+ RTE (40%) and mature Thy-1- antigen-experienced T cells (60%) (45) derived from the CD45RC+ subset (32, 38). To avoid ambiguity, RTE were always removed from the CD45RC+ subset during purification.

Using T cell subsets purified from TDL of unprimed rats, the DNCB response was transferred to nude recipients by the CD45RC+ subset alone, as expected; the CD45RC- subset from unprimed rats lacked T cells specific for DNCB (Fig. 1 a). To show that specific antigen altered isoform expression, CD45RC+ and RC- subsets were purified from TDL of DNCB-primed rats 4 d after sensitization, a time at which ear-challenge would normally induce immediate ear swelling. Fig. 1 b shows that the DNCB response was now transferred with the CD45RC+ subset alone. Note, however, that in contrast with the experiment in Fig. 1 a, nude recipients in Fig. 1 b were ear-challenged immediately (instead of at 3 wk) after cell transfer, a protocol that also demonstrated that the CD45RC+ subset was responsible for the effector stage of the response. Although recently stimulated, the DNCB-responding T cells were not in a state of hyperacute activation. For example, the purified CD45RC- subset displayed size and granularity characteristics that were indistinguishable from small lymphocytes (15). Furthermore, depleting the CD45RC- subset of OX40+ cells, a marker of activated T cells in the rat (36), did not significantly reduce the adoptively transferred DTH response (Fig. 1 b). This evidence is compatible with the notion that the low molecular weight isoform of CD45RA marks memory T cells (20-24, 32, 33).

Antigen-specific CD45RC- CD4 T cells revert to CD45RC+. Investigations based on whole cell populations, but with no regard for antigen specificity, have indicated that CD45RC- T cells (and comparable CD45RA-RO+ T cells in humans) will reexpress with time the high molecular weight isoform (CD45RC+ in rats; CD45RA+ in humans) both in vitro (46-49) and in vivo (27-29, 34, 45). However, there is no clear demonstration that such reversion (from a memory to a naive phenotype) will occur within an antigen-specific population in vivo. Therefore, we determined whether DNCB-specific memory T cells remained CD45RC- or could revert to CD45RC+ with time. To exclude a contribution of newly generated naive CD45RC+ CD4 T cells from the thymus, animals were thymectomized (Fig. 1 a). Over half the DNCB-specific activity was now found within the CD45RC+ subset (Fig. 2 b), suggesting that antigen-specific T cells had switched back from a CD45RC- state. We were also clear that at 2 mo, the CD45RC- subset still retained a large population of DNCB-specific T cells.

This experiment does not exclude the possibility that the response generated by the CD45RC+ subset at 2 mo could have originated from residual naive T cells not stimulated during the primary sensitization. We asked, therefore, how efficient was the sensitization in converting naive T cells to a CD45RC- phenotype. At day 4 after sensitization CD4...
T cells were separated into CD45RC⁺ and RC⁻ subsets and transferred to nude recipients (Fig. 2 d), but not immediately challenged (unlike the experiment in Fig. 1 b). 2 mo later the reconstituted nude recipients were sensitized and ear-challenged. The results demonstrated that virtually all the DNCB-specific activity at day 4 after priming was confined within the CD45RC⁺ subset (Fig. 2 d); five times as many CD45RC⁺ CD4 T cells failed to transfer a significant DTH response. Thus, by day 4, the CD45RC⁺ subset was essentially devoid (clonally depleted) of DNCB-specific T cells. Hence, the reappearance of DNCB activity within the CD45RC⁺ subset at 2 mo strongly suggested that specific T cells reverted from the CD45RC⁻ subset.

To investigate more conclusively whether antigen-experienced memory T cells can revert from CD45RC⁺ to RC⁻, highly purified CD45RC⁻ CD4 T cells (>98.0% pure) from 4-d sensitized rats were “parked” in athymic nude recipients until retransfer (Fig. 3, left protocol). Approximately 60% of the donor-derived population (carrying the RT7b allotype marker) subsequently re-expressed the high molecular weight isoform (CD45RC⁺) (e.g., 34, 35). Donor-derived CD45RC⁺ revertants and CD45RC⁻ CD4 T cells were recovered from TDL of the intermediate nude rats after 2 mo, purified and re-transferred to secondary nude recipients. Surprisingly, the DNCB-specific response was contained entirely within the CD45RC⁺ subset (Fig. 3 a). No specific DTH activity remained within the CD45RC⁻ subset; an equal number of retransferred CD45RC⁻ CD4 T cells gave a response that was indistinguishable from the negative control. Clearly antigen-specific memory T cells survived in the complete absence of antigen, but all expressed the CD45RC⁺ phenotype.

Because the switch-back was so decisive, we asked whether the DNCB-specific activity remaining in the CD45RC⁻ subset of the previous experiment (Fig. 2 a) could have been caused by persisting antigen. To test this idea, CD45RC⁻ CD4 T cells (>98.5% pure) were transferred into intermediate nude recipients as before, except that the animals were ear challenged on the day of transfer. The T cell–injected nude rats were those from the experiment in Fig. 1 b; they received no other source of antigen. 7 mo later donor-derived CD4 T cells were recovered from TDL, purified into CD45RC⁺ and RC⁻ subsets, and transferred into secondary nude recipients. After 3 wk,
these animals were sensitized and ear challenged in the standard way (Fig. 3, right protocol). Again, a majority of antigen-experienced memory T cells reverted to a CD45R C+ phenotype. However, a significant number of DNCB-specific T cells were still found within the CD45R C- subset. This suggests that residual antigen was preventing antigen-experienced CD45R C- T cells from defaulting to a CD45R C+ state. Furthermore, the results indicate that there are two distinct categories of memory CD4 T cells, one CD45R C- the other CD45R C+.

CD4 T Cell Infiltration. The ears of DNCB challenged nude recipients were examined immunohistochemically for the presence of donor-derived CD4 T cells, i.e., those staining positive for the RT7b allotype marker. Representative samples from the experiment in Fig. 3 a are illustrated in Fig 4. The ears of uninjected nude controls, which showed no net increase in ear thickness, were devoid of allotype-marked cells. In contrast, recipients' ears of CD45R C+ revertants were heavily infiltrated; donor T cells were abundant in the greatly thickened dermis and epidermis. Curiously, all samples in this group had numerous consolidated foci on the outer surface of the epidermis containing dense collections of polymorphonuclear leukocytes. The increased ear thickness, induced by the CD45R C+ revertants and measured in situ, was clearly visible in cross section (Fig. 4, insets). The ears of nude rats injected with nonresponsive CD45R C- CD4 T cells, lacking DNCB-specific T cells, were also infiltrated with donor-derived CD4 T cells, but to a lesser extent. Clearly, both DNCB-specific and non-specific CD4 T cells had ready access to the ear, but only the former induced changes (presumably by cytokine release) that caused a local increase in ear thickness.

Response of CD45R C- CD4 T Cell Revertants. An important question arises as to whether CD45R C+ revertants display characteristics similar to or different from truly naive antigen-inexperienced CD45R C- T cells. Available evi-
DNCB-specific CD45RC 

Although not proven, this early increase could be due to response (CD45RC 

collection from 2-mo primed thymectomized rats, transferred unprimed T cells. For example, when revertants were col-

Discussion 
The main conclusions of the present investigation were (a) that antigen-specific CD4 T cells, which expressed a low molecular weight isoform (CD45RC^-) after antigen encounter, reexpressed the high molecular weight isoform (CD45RC^+) in large numbers, (b) that, in the absence of antigen, the switch back was total and (c) that persisting antigen could prevent reversion. Since both CD45RC^+ and R C^- subsets represent functionally distinct populations, the results indicate that there are two major types of antigen-experienced CD4 memory T cells.

CD4 T cells are pivotal in the immune response in that they control the development and activation of B cells, CD8 T cells, macrophages and other inflammatory cells. Assessing the response of memory CD4 T cells in vivo is consequently indirect (antibody production, cytotoxicity, intra-

Figure 4. Donor-derived CD4 T cells infiltrate the ears of DNCB-challenged, T cell reconstituted nude rats. Ears from uninjected nude rats (control) or from nude rats injected with CD45R C^- revertants or with unresponsive CD45R C^- T cells (all taken from rats of experiment Fig. 3a at 48 h) were snap frozen, sectioned, and stained for the presence of RT7^a allotype-marked donor cells as described elsewhere (50). There was a heavy infiltrate of donor-derived CD4 T cells in the dermis (de) and epidermis (ep) of recipients reconstituted with CD45R C^- revertant T cells and the surface of the epidermis showed necrotic foci (*) containing numerous polymorphonuclear leukocytes. In cross section, the ears were swollen, more cellular, and increased in thickness compared with ears from control and CD45R C^- T cell-injected nude rats (inserts). The ears of the latter recipients showed a modest infiltrate of donor-derived cells but were not swollen. Original magnification ×80; inset ×10. cu, cutis; ca, cartilage; mu, muscle. The immunostaining and photographs were kindly provided by K. Bankes-John and Jürgen Westermann, Hannover, Germany.

cellular killing, inflammation, infiltration, swelling). Although primary sensitization occurs centrally within draining LNs (51, 52), we presume that the ear swelling measured here is the culmination of factors released by antigen-specific CD4 T cells exposed locally to DNCB. A number of studies in mice have shown that contact sensitivity is mediated by CD4 T cells (39, 53), although recent investigations have linked the effector stages with CD8 T cells (54). In our experiments the effector stage of the response was transferred to nude rats by antigen-experienced CD45R C^- CD4 T cells and donor-derived CD4 T cells were identified in large numbers within the inflamed ears (Fig. 4). We have not rigorously excluded a role for CD8^+ T cells, but, if involved, they must be of extrathymic origin (55) and subservient to CD4 T cells.

In unprimed naive rats, DNCB-specific CD4 T cells were found only within the CD45R C^- subset; the CD45R C^- compartment was devoid of reactivity. In contrast, after acute sensitization, DNCB-primed T cells were concentrated in the CD45R C^- population. Neither of these observations was unexpected and confirm what others have shown (9, 18, 32, 33). What has not been highlighted before was the completeness of the isoform switch. It was known from earlier work that antigen rapidly recruited specific lymphocytes into secondary lymphoid tissues, effectively removing them for a period of time from the recirculating pool (56-58). Our evidence suggested that recruitment was highly efficient and that the entire DNCB-specific population switched from CD45R C^- to RC^- by day 4 after sensitization. Consequently, the CD45R C^- subset became negatively selected, devoid of DNCB-specific T cells. Nude recipients injected with this depleted CD45R C^- subset never developed a DNCB response, even many months after reconstitution.

One of the primary aims of the investigation was to determine whether antigen-specific T cells, once induced to express the low molecular weight isoform, returned to a CD45R C^+ naive phenotype. That DNCB-specific CD45R C^- CD4 T cells did revert was shown in two dif-
cells revert to a CD45RA state. In practice the organism may never rid itself of residual antigen. Others have hypothesized that once primed, T cells may be readily activated and could be maintained in a CD45Rlow memory state by restimulation with low affinity cross-reacting antigens (59). The fact that the DNCB-specific population reverted in its entirety to a naive phenotype indicates that, at least for this antigen, putative cross-reacting antigens played no demonstrable role. Given that specific antigen was found to exert a long-term effect, it is not necessary to hypothesize a role for cross-reacting antigen.

Earlier evidence from whole populations indicated that isoform exchange was cyclic (28). Thus, a switch between CD45R C+ and RC− could occur more than once, perhaps governed by chance encounter with retained antigen. This proposed pattern of events coincides with the known turnover of the two subsets (12, 28). The CD45R Bhigh subset in mice was shown to be comprised largely of long-lived cells in interphase, whereas a majority of the CD45R Blow fraction was rapidly dividing and incorporated Brdu (12). A small but significant fraction of resting CD45R Bhigh cells was also labeled with Brdu (12, 60). The present results would indicate that the labeled CD45R Bhigh cells were likely to be revertants, rather than "T cells which divided without losing their naive phenotype" (12). Life span studies in humans based on cells bearing radiation-induced dicentric chromosomes (27, 29) and in rats after the loss of transferred allotype-marked CD4 T cells (28) both reached the same conclusion: CD45RA+(human) or CD45RC+ (rat) T cells were long-lived; CD45RA− (CD45R O+) or CD45RC− T cells were short-lived. It is important to note that turnover of human lymphocytes was measured in months, not days as in rodents, reflecting the considerable difference in life span between these species.

If CD45R low T cells were to be the sole purveyors of memory, it becomes difficult to reconcile the long duration of functional memory in vivo with the known characteristics of the CD4 CD45R low subset. We think the present observations resolve this paradox. There are two types of CD4 memory T cells (a) memory T cells (CD45R low) that respond quickly (24), upregulate adhesion molecules, migrate differently (13-15, 21) but survive less well (12, 27-31) and (b) the antigen-experienced revertants that regain a full resting state and migrate conventionally but endure. The former contributes the qualitative attribute (speed) of the classical memory response, the latter only the quantitative element. This idea (35) has also been supported by others (61).

What, therefore, is the nature of the CD45R C+ revertants? Have they been changed by their previous encounter with antigen? We have been unable to find any phenotypic or functional characteristics that distinguish the revertants from CD45R C+ CD4 T cells that have never seen antigen. For example CD45R C+ revertants regain their ability to induce graft-versus-host responses (34, 46). CD44 and L-selectin (CD62L), markers associated with activated CD8 memory T cells (6, 9, 10, 18, 19), were not decisive in the rat. CD44 expression in the rat was not significantly upregulated.
lated in the CD45R C−CD4 T cell subset and was similar in this regard with certain mouse strains (11). With respect to L-selectin, essentially all CD45R C+CD4 T cells, which would include revertants, and two thirds of the CD45R C−subset were L-selectin-high (mAb HRL-1 and HRL-2 [62]). The populations of CD45R C−T cells defined by high and low expression of L-selectin are under investigation. A detailed assessment of mRNA encoding splice variants of CD45R (using reverse transcription and the polymerase chain reaction) showed that revertants were identical with truly naive CD4 T cells, generated from recent thymic emigrants (Hargreaves, M., and E.B. Bell, manuscript submitted for publication). When DNCB-primed rats were re sensitized, the kinetics of the new response were the same as those in once-primed animals (Fig. 5). However, we do not know whether the revertant T cells remained committed to the cytokine profile (Th1 or Th2) induced by DNCB. Others reported that the cytokine patterns of resting memory CD4 T cells were permanently polarized (22). However, the antigen-primed transgenic T cells used in that study remained CD45R Blow and never returned to the CD45RBhigh antigen and clonally expanded. Recent experiments (Bunce, C., and E.B. Bell, unpublished) showed that DNCB-specific CD4 T cells increased three- to fivefold among the CD45R C− revertant population. An increase in frequency of antigen-specific T cells after antigen stimulation was clearly documented for CD8 T cells (63–65) and many of these down-regulated activation molecules with time (9, 18, 19).

The present results afford a new perspective on the role of persisting antigen in the maintenance of immunological memory. Persisting antigen ensures an accelerated response by CD45R low T cells and the ability of these to migrate into non-lymphoid sites; qualities important for functional protection against, e.g., viruses (9, 65). Should antigen disappear entirely, memory T cells simply revert to a quiescent state and decay more slowly; for some infections, quantity may be more important than speed. Immunity is not, of course, always permanent and failure to protect could be a consequence of T cells merely downregulating their activation state or be due to the eventual loss of the CD45R high resting population itself. We suggest, therefore, that the amount of persisting antigen could apportion the distribution of specific T cells between the two subsets and determine the precise character of the secondary response, balancing the desirability of rapid onset against the need to retain a high frequency of specific cells with which to combat an infrequent antigenic insult.

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