THE MRC OX-22$^-$ CD4$^+$ T CELLS THAT HELP B CELLS IN SECONDARY IMMUNE RESPONSES DERIVE FROM NAIVE PRECURSORS WITH THE MRC OX-22$^+$ CD4$^+$ PHENOTYPE

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In man, rat, and mouse it has been shown that CD4$^+$ T cells can be divided into two subpopulations on the basis of their reactivity with mAbs that recognize a restricted number of members of the leukocyte common antigen (CD45) family of glycoproteins (1–4). Assays of T cell function carried out on the two subpopulations have shown that this phenotypic heterogeneity is associated with a functional one and data obtained in the three species, while not completely concordant, are substantially in agreement (5).

In the rat the mAb MRC OX-22, which binds to some of the higher molecular mass forms of CD45 (6) but not to the low (180 kD) molecular mass one, labels ~70% of CD4$^+$ T cells. Assays of T cell function have shown that it is the MRC OX-22$^+$ CD4$^+$ T cells that proliferate most vigorously to allogeneic stimulator cells and to T cell mitogens (7) and produce high levels of IL-2, whereas the MRC OX-22$^-$ CD4$^+$ cells provide the majority of help for B cells in secondary immune responses, both in vivo and in vitro (1, 7).

It has been the source of much debate whether these two major subpopulations of CD4$^+$ T cells represent reciprocal, nonoverlapping subsets of mature cells with distinct functions or that the subsets represent different stages of T cell maturation. In man, CD4$^+$ T cells isolated from umbilical cord blood do not contain the subset that expresses the low molecular weight form of the CD45 antigen, whereas this population is present in adult peripheral blood and contains within it those cells that respond to recall antigens such as mumps virus and tetanus toxoid (2, 8). Furthermore, on in vitro stimulation, CD4$^+$ T cells expressing the higher molecular mass forms of CD45 change their phenotype and come to express only the 180-kD form of the antigen, i.e., they acquire the characteristics of the subset of T cells that display immunological memory (9). These observations, while giving support to the view that the two subsets of CD4$^+$ T cells are related by lineage, do not exclude the possibility that CD4$^+$ T cells that respond to recall antigens derive from cells of a separate lineage that also expresses only the low molecular weight forms of the CD45 antigen.

In our earlier work attempts were made to establish a lineage relationship between the MRC OX-22$^+$ CD4$^+$ (putative precursor) and MRC OX-22$^-$ CD4$^+$ (product) subsets of rat T cells by priming rats with allogeneic skin grafts and testing the MRC OX-22$^-$ cells for alloreactivity in vitro. No evidence of T cell priming could be obtained in these experiments (7). Furthermore, adult thymectomy carried

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out to cut off the source of fresh precursors failed to demonstrate a decline in the size of the putative precursor pool (7).

These experiments failed to support the view that the two subsets represented different stages of T cell maturation but the essentially negative nature of the results made a definite conclusion impossible (7). Given these uncertainties and the lack of a concensus view on the situation in man, further study of the relationship between the two subsets of $CD4^+$ T cells was clearly indicated.

Herein, we describe in vivo experiments designed specifically to establish the lineage relationship between the two subsets of $CD4^+$ T cells.

Materials and Methods

Rats. The PVG.RT1c rats used were from the specific pathogen-free (SPF)[1] unit of the Medical Research Council Cellular Immunology Unit (Oxford, UK). Congenitally athymic nude rats (PVG rnu/rnu) were obtained from Harlan Olac (Bicester, Oxon) and maintained under SPF conditions throughout the course of the experiment. Irradiations were performed using a ^{137}Cs source at 94 rad/min (Gammacel; Atomic Energy of Canada Ltd., Kanata, Ontario).

Preparation of Adult Thymectomized, Lethally-irradiated, Bone Marrow-restored Rats (B Rats). PVG rats were thymectomized at 6–8 wk of age. 2 wk later the animals were irradiated with two 600-rad doses of γ radiation 3 d apart, immediately after which, the animals were restored with 10^7 T cell-depleted bone marrow cells from syngeneic donors.

Cells. Thoracic duct lymphocytes (TDL) were obtained by cannulation of the duct (10). Cells from a pool of at least three PVG.RT1c rats were collected at 4°C overnight into flasks containing PBS and 20 U/ml heparin.

Antibodies. The derivation of the mouse mAbs used in this work, viz W3/25 (anti-CD4) (11), MRC OX-8 (anti-CD8) (11), MRC OX-12 (anti-rat k chain) (12), MRC OX-6 (anti-rat class II) (13), MRC OX-39 (anti-rat IL-2R) (14). MRC-OX-40 (against a cell surface antigen on $CD4^+$ T cell blasts) (14). MRC OX-22 (1), and MRC OX-21 (15), have all been described in the references given. Biotinylated mAbs were prepared as described (16).

Isolation of Subsets of Lymphocytes. Subpopulations of TDL were prepared by negative selection using the rosetting technique (16), and cell fractionation was also carried out by means of a FACS II (Becton Dickinson Immunocytometry Systems, Mountain View, CA) as previously described (7). For all fractionation procedures purity was assessed by FACS analysis.

Reconstitution of T Cell-deficient Rats with T Cell Subpopulations. Subpopulations of TDL were injected intravenously into 6–10-wk-old nude or B rats. Animals also received 100–500 μg of alum-precipitated OVA or dinitrophenyl-conjugated OVA (DNP-OVA) intraperitoneally together with 10^9 killed *Bordetella pertussis* organisms at the time of T cell reconstitution.

FACS Analysis of TDL from T Cell-Replaced Nude and B Rats. 8–10 weeks after T cell reconstitution 5×10^6 TDL were analyzed by two-color immunofluoresence on the FACS as described (16).

Binding Assay for Specific Antibody. Rat sera were diluted in PBS containing 0.2% BSA (PBS/0.2% BSA) and 50 μl aliquots were assayed in duplicate for anti-OVA or antihapten antibodies using a solid phase RIA, essentially as described in reference 1, except that the developing antibody used was ^{125}I-OX-12 $F(ab')_2$ and for anti-OVA assays plates were prepared by coating with 100 μl per well of a 100-μg/ml solution of OVA in PBS containing 0.5 mM NaN_3, at 4°C overnight.

Results

Phenotype of Lymphocytes Recovered from T Cell-deficient Rats Reconstituted with Subsets of $CD4^+$ T Cells. When $CD4^+$ T cells are injected into nude recipients, the donor

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[1] *Abbreviations used in this paper:* SPF, specific pathogen free; TDL, thoracic duct lymphocytes.

T cells undergo extensive proliferation leading to a marked increase in cell numbers (17, 18). This system was used to test the ability of the MRC OX-22$^+$ and MRC OX-22$^-$ subsets of CD4$^+$ T cells to expand in vivo and in particular to determine whether the progeny of the MRC OX-22$^+$ CD4$^+$ inoculum retained the donor cell phenotype or became MRC OX-22$^-$ CD4$^+$.

Irrespective of whether nude or B rats were used as recipients, T cell subsets underwent a 100–1,000-fold expansion in the recipient animals as judged by the frequency of T cells in thoracic duct lymph (Table I). In the case of the MRC OX-22$^+$ CD4$^+$ T cell inoculum, the recipients were restored to near normal CD4$^+$ T cell levels. However, these animals were far from normal in that there were very few detectable MRC OX-22$^+$ CD4$^+$ T cells (Table I). The great majority of CD4$^+$ T cells in these animals were of the MRC OX-22$^-$ phenotype. That these MRC OX-22$^-$ CD4$^+$ T cells derived from the expansion of the MRC OX22$^+$ CD4$^+$ cells and not from a very minor contamination of MRC OX-22$^-$ CD4$^+$ T cells is supported by the fact that TDL from rats given much larger doses of purified MRC

TABLE I

Phenotype of TDL from T Cell-replaced B Rats or Athymic Rats 8–10 wk after Reconstitution

	Percent positive cells (of the total)						
T cells injected	CD4$^+$	CD4$^+$ OX22$^-$	CD4$^+$ OX22$^+$	CD4$^+$ IL2R$^+$	CD4$^+$ OX40$^+$	sIg$^+$	OX8$^+$
1–5 × 10^6 CD4$^+$	25, 21*	21, 20	3, 2.7	7, 10	ND, 14	74, 67	2, 2
1–5 × 10^6 CD4$^+$ OX-22$^+$	41, 48	38, 45	3, 1.3	12, 23	22, 37	56, 43	2, 2
1–5 × 10^6 CD4$^+$ OX-22$^-$	17, 11	14, 10.5	3, 1.6	3, 6	3, 6	82, 79	2, 3
Nude or B rat	5, 3	5, 2	1, 1	1, 1	1, 1.4	90, 83	2, 2
Normal PVG	39	14	23	1	1	51	7

CD4$^+$ T cells were isolated from PVG TDL by removing MRC OX-8$^+$, MRC OX-6$^+$, and MRC OX-12$^+$ cells by rosette depletion as described in reference 7; recovered cells were then kept on ice at 4°C or labeled with MRC OX-22 antibody and sorted on the FACS into positive and negative fractions. Purities of sorted cells were >98% for both populations. T cell-deficient rats were reconstituted with 1–5 × 10^6 fractionated PVG CD4$^+$ T cells. 8–10 wk later the recipients were cannulated and the TDL collected overnight were phenotyped by two-color immunofluorescence on a FACS, using biotinylated W3/25 mAb in combination with other mAbs.

Where pairs of numbers are given, the first refers to data from B rats and the second from nudes. Data are the mean value obtained from three animals taken from two experiments. Reaction with MRC OX-21 mAb that binds to a component of human complement, but not rat tissues, was used as a negative control, and in all cases values obtained were <0.6%. Ranges are not shown as they were within 10% of the mean value for all determinations. The range of TDL output in 18 h is given in parenthesis: B rats reconstituted with; CD4$^+$ T cells (1.0–3.3 × 10^8), MRC OX-22$^+$ CD4$^+$ T cells (0.43–1.98 × 10^8), MRC OX-22$^-$ CD4$^+$ T cells (1.34–1.76 × 10^8), and unreconstituted B rats (0.2–2.0 × 10^8). Nude rats reconstituted with: CD4$^+$ T cells (1.1–2.7 × 10^8), MRC OX-22$^+$ CD4$^+$ T cells (0.6–2.3 × 10^8), MRC OX-22$^-$ CD4$^+$ T cells (1.9–3.3 × 10^8), and unreconstituted nude rats (2.3–4.95 × 10^7). Normal PVG rats gave cell yields in the range, 2–3.8 × 10^8. As the above data indicate some of the experimental groups showed large variations in TDL output but the results of several independent experiments revealed that the surface phenotype of the TDL obtained was insensitive to the dose of the T cell inocula or the TDL output.

OX-22$^-$ cells contained a lower frequency of T cells than did the recipients of the MRC OX-22$^+$ T cell subset (Table I). In fact, the MRC OX-22$^-$ T cell population expanded in vivo to a similar level to that found in normal TDL, i.e., ~12%. It is known that the expression of the MRC OX-22 epitope is lost on T cell activation in vitro (14), but it has not been shown whether it is reexpressed when T cells return to a resting state. These in vivo data confirmed the in vitro results showing loss of the epitope on T cell activation but also showed that the antigen is not reexpressed when T cells are no longer activated. Some of the CD4$^+$ T cells recovered from nude rats (25%) given MRC OX-22$^+$ CD4$^+$ T cells had reverted to a resting state, as indicated by the fact that they did not express IL-2-Rs or the MRC OX-40 determinant (a CD4$^+$ blast antigen [14]) but they remained phenotypically MRC OX-22$^-$ CD4$^+$. B rats injected with MRC OX-22$^+$ CD4$^+$ T cells had an even higher frequency of MRC OX-22$^-$ CD4$^+$ T cells with the characteristics of resting cells (50%).

Phenotype of the T Cell Reactive in Primary Immune Responses In Vivo. Immunization of nude rats with DNP-OVA at the time of T cell reconstitution resulted in significant antihapten antibody responses in the sera of animals given CD4$^+$ T cells or the MRC OX-22$^+$ fraction thereof (Fig. 1). The MRC OX-22$^-$ CD4$^+$ reconstituted animals failed to make a response above that of the unreconstituted nude negative control. Titration of sera obtained 20 d later gave similar results (data not shown). In this experiment the dose of T cells given was in physiological ratios (i.e., approximately three times as many MRC OX-22$^+$ cells as MRC OX-22$^-$ cells). In a second experiment using B rats as T cell recipients, equal T cell doses were given. Measurements of the anti-OVA antibody response at 21 d (not shown) and 40 d (Fig. 2) also showed that it was only the recipients of MRC OX-22$^+$ CD4$^+$ or unseparated CD4$^+$ T cells that produced antibody.

Previous data from this laboratory, obtained using adoptive transfer into acutely preirradiated recipients, have shown that T cells that provide B cell help for secondary immune responses to haptenated protein are of the MRC OX-22$^-$ CD4$^+$ phenotype (1). In contrast, these new results, derived from chronically T cell-deficient hosts, show that it is T cells that belong to the MRC OX-22$^+$ CD4$^+$ population that provide help for a primary antibody response to a soluble antigen in vivo. To

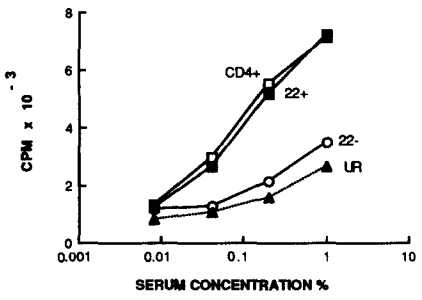

FIGURE 1. Primary antihapten antibody response in T cell-reconstituted nude rats. CD4$^+$ T cells were isolated from PVG TDL and separated into MRC OX-22$^+$ and MRC OX-22$^-$ fractions as described in Table I. Nude rats were reconstituted with 5×10^6 MRC OX-22$^+$ CD4$^+$ ($n = 3$), 5×10^6 CD4$^+$ ($n = 3$), or 1.5×10^6 MRC OX-22$^-$ CD4$^+$ ($n = 2$) T cells. Two animals were not given cells. At this time all of the animals were immunized with 500 μg of DNP-OVA, as described in Materials and Methods. The data represent the mean value of duplicate determinations of the antihapten antibody, as assessed by a solid phase RIA on DNP-BGG-coated plates, in the pooled sera of each experimental group, at 14 d in the response. The sera were also titrated individually and the range of values was shown to lie within 5% of the mean value for all points in all experimental groups. Purities of separated CD4$^+$ T cells were >98%.

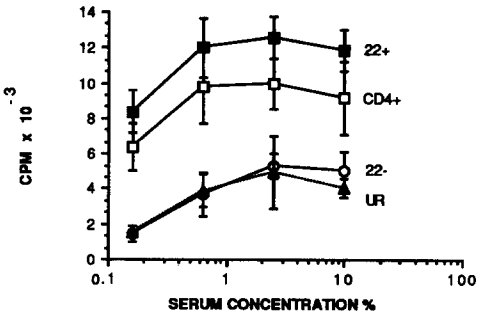

FIGURE 2. Primary anti-OVA antibody response in B rats reconstituted with subpopulations of $CD4^+$ T cells. 2×10^6 MRC $OX\text{-}22^+$ or MRC $OX\text{-}22^-$ fractions of $CD4^+$ TDL, purified as outlined in Table I, were injected into syngeneic B rat recipients. Control rats received 2×10^6 CD4+ T cells, or were not reconstituted at all. At the time of cell transfer all recipients were immunized with 100 μg of OVA. The anti-OVA response was determined 40 d later by solid phase RIA on OVA-coated plates. Purities for the separated $CD4^+$ T cells were >99%. The results represent means with ranges for three rats per group for T cell-reconstituted animals and two rats per group for the unreconstituted animals.

ensure that the MRC $OX\text{-}22^-$ $CD4^+$ T cells were not incapable of responding in this latter system, a test was made of the ability of these cells to provide help for secondary immune responses.

B rats were reconstituted with $CD4^+$ T cell subsets obtained from primed donors and challenged with specific antigen at the time of T cell reconstitution. Table II shows that both T cell subsets provided help for antihapten antibody responses when these were assayed 28 d later, illustrating that MRC $OX\text{-}22^-$ T cells were perfectly capable of providing help for a secondary immune response in T-deficient rats. It is notable that the MRC $OX\text{-}22^+$ T cells provided equally good help, which suggests that on priming of the T cell donors not all of the antigen-reactive T cells moved into the memory pool. This point will be pursued further in the discussion.

MRC $OX\text{-}22^-$ Memory T Cells Derive from MRC $OX\text{-}22^+$ Naive Precursors. Given that memory T cells are of the MRC $OX\text{-}22^-$ phenotype, the question arises as to whether these cells originate from MRC $OX\text{-}22^+$ $CD4^+$ precursor cells, the population in which primary T helper reactivity resides, or whether they derive as a separate lineage, from an MRC $OX\text{-}22^-$ $CD4^+$ precursor. To address this point, B rats, reconstituted several months earlier with subpopulations of $CD4^+$ T cells from

TABLE II

Secondary Antihapten Antibody Responses in B Rats Reconstituted with Subsets of $CD4^+$ T Cells from Antigen-primed Donors

Reconstitution	Percent relative antibody titer*
1.5×10^6 $CD4^+$ T cells	100
1.5×10^6 MRC $OX\text{-}22^+$ $CD4^+$ T cells	102.5
1.5×10^6 MRC $OX\text{-}22^-$ $CD4^+$ T cells	87.5
Unreconstituted	12.5

$CD4^+$ T cells were isolated from rats primed 8 wk earlier with 100 μg alum-precipitated DNP-OVA, and separated into MRC $OX\text{-}22^+$ and MRC $OX\text{-}22^-$ fractions as described in Table I. B rats were reconstituted with 1.5×10^6 T cells and immunized with 100 μg DNP-OVA. Sera were assayed 28 d later for antihapten antibodies by a solid phase RIA on DNP-BGG-coated plates.

* The mean antihapten antibody titers are expressed as a percentage of the control response, produced by unfractionated $CD4^+$ T cells. There were two animals per group. Purities of separated $CD4^+$ T cells were >98%.

normal donors, and immunized at the time of cell transfer, were challenged with the same antigen to see whether an anamnestic response could be elicited.

Several of the animals used to generate the primary anti-OVA responses shown in Fig. 2 were kept for 5 mo, at which time their anti-OVA titers were measured. The MRC OX-22$^+$ CD4$^+$ and unseparated CD4$^+$ T cell-reconstituted animals still showed good antibody titers (Table III), whereas the MRC OX-22$^-$ CD4$^+$ T cell-reconstituted animals still failed to show a significant antibody titer. Challenge with antigen at this time led to a considerable increase in antibody titer in animal's number 4 (MRC OX-22$^+$ T cell reconstituted) and number 3 (CD4$^+$ T cell reconstituted). A smaller increase in antibody titer was seen in animal number 8 an MRC OX-22$^-$ T cell-reconstituted animal (Table III). This latter finding may represent antigen-reactive T cells that were originally MRC OX-22$^+$ and present as a small contamination in the original T cell inoculum which, after 5 mo expansion in vivo, could provide some B cell help. It is notable that the secondary anti-OVA titer from this MRC OX-22$^-$ T cell-reconstituted animal is 100-fold lower than the secondary response evoked in the MRC OX-22$^+$ T cell-reconstituted animal. This was not the case with the other MRC OX-22$^-$ CD4$^+$ T cell-reconstituted animal, number 9, or the unreconstituted negative control animals numbers 10 and 11, which showed no increase in antibody titer upon antigenic challenge, suggesting there were no antigen-reactive T cells in these animals.

A further experiment showed that 10^7 TDL obtained from a B rat donor reconstituted 12 wk earlier with MRC OX-22$^+$ CD4$^+$ T cells (one of the animals from the experiment described in Fig. 2, but not used in that described in Table III) could adoptively transfer B cell help for anti-OVA responses into sublethally irradiated syngeneic recipients, whereas TDL obtained at this time from animal number 9 (Table III) could not (Fig. 3). As this acute adoptive transfer system requires that, in order to get an antibody response to soluble antigen in the recipient animals, the donor lymphocytes must be primed (1), one can conclude that MRC OX-22$^-$

TABLE III

Induction of Secondary Immune Responses in B Rats Reconstituted with Subsets of CD4$^+$ T cells

Animal No.	Cell dose	Primary titer	Secondary titer	Increase (primary to secondary)
		%	%	
2	2 × 10^6 CD4$^+$ T cells	40	80	2×
3	2 × 10^6 CD4$^+$ T cells	16	312.5	20×
4	2 × 10^6 OX-22$^+$ CD4$^+$ T cells	44	704	16×
8	2 × 10^6 OX-22$^-$ CD4$^+$ T cells	1.5	7.5	5×
9	2 × 10^6 OX-22$^-$ CD4$^+$ T cells	<1.5	<1.5	0
10	Unreconstituted	<1.5	<1.5	0
11	Unreconstituted	<1.5	<1.5	0

B rats reconstituted and immunized as outlined in Fig. 2 were kept for 5 mo, at which time their sera were assayed for anti-OVA antibodies. They were then challenged with 100 μg OVA intravenously and their sera were titrated 7 d later. Serial dilutions were assayed in duplicate. Data represent the titer expressed as a percentage of the value obtained with a reference antiserum to OVA raised in PVG rats.

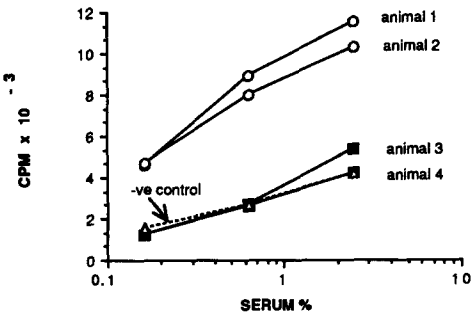

FIGURE 3. Adoptive transfer of a secondary anti-OVA antibody response with cells that originated from the OX-22$^+$ CD4$^+$ T cell population. 10^7 TDL were obtained from B rat donors that had been T cell reconstituted and immunized 3 mo earlier as described in Fig. 2. These cells were adoptively transferred into sublethally irradiated (750 rad) syngeneic recipients. Animals 1 and 2 received TDL obtained from a donor animal that had originally received MRC OX-22$^+$ CD4$^+$ T cells, whereas animals 3 and 4 received TDL from an MRC OX-22$^-$ CD4$^+$ T cell-restored donor. At the time of T cell transfer, the recipients were immunized with 100 μg OVA intravenously. Sera obtained 9 d later were titrated for anti-OVA antibody using a solid phase RIA. Data represent duplicate determinations. The negative control serum used was normal PVG serum.

CD4$^+$ memory T cells active in secondary responses, like those responsible for B cell help in primary responses, derived from T cells that belong to the MRC OX-22$^+$ CD4$^+$ population, and not from a putative separate cell lineage that is MRC OX-22$^-$ before exposure to antigen.

Discussion

The in vivo T cell reconstitution studies described here demonstrate a precursor-product relationship between MRC OX-22$^+$ and MRC OX-22$^-$ subsets of CD4$^+$ T cells involved in the provision of helper activity for B cells. The experiments show that in assays of primary B cell help, the MRC OX-22$^+$ CD4$^+$ T cells were active in contrast to assays of secondary help, in which the MRC OX-22$^-$ CD4$^+$ T cells were the most potent (1). Further, the antigen-specific memory T cells in the MRC OX-22$^-$ CD4$^+$ population derive from MRC-OX-22$^+$ CD4$^+$ precursors.

Consistent with this lineage relationship between the two subsets of CD4$^+$ T cells is the finding that nude or T cell-deficient B rats reconstituted with CD4$^+$ or MRC OX-22$^+$ CD4$^+$ T cells contain, 8–10 wk after T cell reconstitution, CD4$^+$ cells that are virtually all MRC OX-22$^-$, even though the animals have reconstituted to near normal CD4$^+$ T cell levels. The stimulus for this expansion of the CD4$^+$ T cells is unknown. It may be that it all derives from environmental antigens to which the T cell-deficient recipient animals have no immunity before cell transfer. The finding that rats primed to a specific antigen had antigen-reactive T cells in both the MRC OX-22$^-$ CD4$^+$ and the MRC OX-22$^+$ CD4$^+$ subsets indicates that the naive T cell pool is not depleted of antigen-reactive cells on priming. It follows that normal rats may be expected to have both memory T cells and naive T cells capable of responding to environmental antigens. However, this hypothesis does not explain the higher frequency of CD4$^+$ T cells in nude or B rats given MRC OX-22$^+$ CD4$^+$ cells compared with recipients of MRC OX-22$^-$ CD4$^+$ T cell inocula. Alternatively, it may be that an antigen-independent expansion occurs, analogous to the autologous MLC in vitro (19), and that this spontaneous activation is inhibited in normal animals by some homeostatic mechanism. Such a mechanism may be expected to maintain T cell numbers at their physiological levels and prevent the improperly regulated

T cell expansion that is observed in T cell–deficient animals injected with relatively low doses of syngeneic T cells. This interesting problem of lymphocyte homeostasis requires further study.

There is no evidence to suggest that the MRC OX-22 epitope is reexpressed in the periphery after it has been lost after T cell activation. Indeed, the data suggest the opposite, that the antigen-reactive daughter cells are MRC OX-22$^-$ even when they revert to a resting phase.

Despite the demonstration that MRC OX-22$^-$ CD4$^+$ peripheral T cells that provide helper activity for B cells derive from MRC OX-22$^+$ CD4$^+$ precursors, acquisition of the MRC OX-22$^-$ phenotype does not simply reflect a numerical expansion of naive cells on encounter with antigen. As stated in the introduction, on activation by T cell mitogen, MRC OX-22$^+$ CD4$^+$ T cells produce much more IL-2 than do cells of the MRC-OX-22$^-$ phenotype and similar results have been found with mouse T cell subsets (4). The data from experiments in man are not clear in this regard. It has been reported that comparable levels of IL-2 can be elicited from CD4$^+$ T cells of naive and memory phenotype (20) but the contrary result, that on activation, naive T cells produce much more IL-2 than memory cells, has also been found (21). Several explanations may be advanced for the difference in IL-2 release between the two subsets of rat T cells. It may be that high levels of IL-2 production are characteristic of CD4$^+$ T cells only on their first encounter with antigen and that the progeny of these high producers become MRC OX-22$^-$ CD4$^+$ cells that have a diminished capacity to synthesize this lymphokine. As the present studies have been carried out on uncloned populations, it is not clear whether the low levels of IL-2 found in activated cultures of MRC OX-22$^-$ CD4$^+$ T cells reflects a low frequency of IL-2 producers or a low level of lymphokine production per cell. Studies on mouse T cell clones have shown that some produce IL-2 while others produce IL-4 (22), and it may be that a similar heterogeneity of lymphokine production occurs with in vitro stimulation of MRC OX-22$^-$ CD4$^+$ T cells obtained directly from normal animals. If so, then it must be concluded that IL-2 producers are not well represented in the MRC OX-22$^-$ CD4$^+$ subset. Further discussion of this important point is complicated by the fact that the human T cell clones apparently fail to fall into two simple categories as described in the mouse (23), in that the majority produce IL-2, and it may be that activated MRC OX-22$^-$ T cells produce varying amounts of IL-2 but that few, if any, produce none.

An alternative explanation for the difference in the level of IL-2 production between the two subsets of CD4$^+$ T cells is that the MRC OX-22$^+$ subset is itself heterogeneous, in that cells that produce high levels of IL-2 on primary activation may retain their MRC OX-22$^+$ phenotype (or fail to become memory cells at all) while those MRC OX-22$^+$ CD4$^+$ cells that, on activation, provide helper activity for B cells change their phenotype to MRC OX-22$^-$ after their primary encounter with antigen. However, the hypothesis that the MRC OX-22$^+$ CD4$^+$ subset contains memory cells for high IL-2 production is not consistent with the observation that in vitro responses to recall antigen cannot be elicited from human T cells that are almost certainly homologous to the MRC OX-22$^+$ CD4$^+$ subset in the rat (2), and similarly, rat T cell lines specific for guinea pig myelin basic protein (J. Sedgewick, personal communication) and clones responding to other soluble proteins and allogeneic stimulator cells (24) have been shown to have the MRC OX-22$^-$ pheno-

type. Consequently, we favor the alternative hypothesis advanced earlier, namely that high levels of IL-2 production are characteristic only of naive $CD4^+$ T cells on their first encounter with antigen and that memory cells produce much less of this lymphokine on activation, at least when these cells are obtained from rats kept in SPF conditions.

If diminished IL-2 production is indeed a characteristic of memory T cells, then the stage in T cell maturation at which this event occurs remains to be precisely defined. Murine T cells that have been deprived of antigenic stimulation for 48 h after primary in vitro activation do not produce significantly lower levels of IL-2 on secondary exposure to antigen (25), so the change in lymphokine production is apparently not acute. It may be that recurrent stimulation of T cells at relatively short intervals can give sustained IL-2 production at high levels, as has been reported for some T cell clones (22).

Summary

$CD4^+$ T cells in the rat can be divided into two nonoverlapping subsets by their reactivity with the mAb MRC OX-22, which binds some of the high molecular weight forms of the CD45 antigen.

The lineage relationship between subsets of $CD4^+$ T cells expression different forms of CD45 has been a controversial issue for some time. Experiments described in this paper address this question using in vivo assays of T cell reactivity.

Analysis of primary antibody responses in vivo show that it is MRC $OX\text{-}22^+$ $CD4^+$ T cells that are active in these assays, whereas antigen-primed T cells that provide helper activity for secondary antibody responses in vivo have the MRC $OX\text{-}22^-$ $CD4^+$ phenotype. It is demonstrated that these memory T cells derive from MRC $OX\text{-}22^+$ $CD4^+$ T cell precursors and not from a putative separate lineage.

It is concluded that with respect to the provision of help for B cells, MRC $OX\text{-}22^+$ $CD4^+$ T cells are precursors of memory cells with the phenotype MRC $OX\text{-}22^-$ $CD4^+$.

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