OX-22^{high} CD4$^+$ T Cells Induce Wasting Disease with Multiple Organ Pathology: Prevention by the OX-22^{low} Subset

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Summary

Congenitally athymic rats injected with CD45RB^{high} CD4$^+$ T cells from congenic euthymic donors developed a severe wasting disease with inflammatory infiltrates in liver, lung, stomach, thyroid, and pancreas. In contrast, recipients of CD45RB^{low} CD4$^+$ T cells remained well and continued to gain weight. Animals given unfractionated CD4$^+$ T cells, i.e., a mixture of approximately two-thirds CD45RB^{high} and one-third CD45RB^{low}, were protected from the wasting disease, and the incidence of organ-specific inflammation was much reduced compared with that found in recipients of CD45RB^{high} cells alone. The data suggest that this latter subset of CD4$^+$ T cells has autoaggressive potential that is inhibited in normal animals by cells of the CD45RB^{low} CD4$^+$ phenotype. The possible consequences of a breakdown in this immunoregulatory mechanism are briefly discussed.

The existence of a variety of autoimmune diseases demonstrates the potential for the immune system to react in a way that is deleterious to the individual. There is also evidence that active immunoregulatory mechanisms exist that normally prevent such immune reactions from taking place. However, the way that these mechanisms mediate their protective role and the cell types involved are poorly understood.

CD4$^+$ T cells in the rat can be divided into two subpopulations by the mAb OX-22,[1] which is reactive with isoforms of the CD45 molecule containing exon B–encoded sequence (1, 2). The phenotypic heterogeneity of CD4$^+$ T cells revealed by this antibody is accompanied by a functional one. OX-22^{high} CD4$^+$ T cells are reactive in assays of GVHD and produce IL-2 and IFN-γ but little IL-4 upon polyclonal activation in vitro. In contrast, the OX-22^{low} CD4$^+$ subpopulation provides the majority of help for secondary antibody production both in vivo and in vitro and is the more potent producer of IL-4 but produces less IL-2 and IFN-γ (1, 3–5). Heterogeneity within the OX-22^{high} CD4$^+$ subset itself is suggested by the recent finding that the OX-22^{low} T cell population involved in the provision of B cell help derives from an OX-22^{high} CD4$^+$ precursor, indicating that, with regard to humoral immunity, a lineage relationship exists between these two subsets (6).

Data described herein demonstrate that the OX-22^{high} and OX-22^{low} subpopulations of CD4$^+$ T cells function interdependently in vivo. Specifically, the OX-22^{low} CD4$^+$ T cells appear to exert an inhibitory action on the OX-22^{high} subset. Injection of small numbers of OX-22^{high} CD4$^+$ T cells into athymic nude rats led to the development of a wasting disease in these recipients 6–10 wk after T cell reconstitution, with immunopathology in several different organs. Animals given the OX-22^{low} subset or unseparated CD4$^+$ cells survived markedly better than recipients of OX-22^{high} cells alone and did not lose weight or show the gross pathological changes seen in the recipients of the OX-22^{high} subset. These data suggest that the T cells, which are the effectors of certain organ-specific autoimmune responses, derive from cells that are phenotypically distinct from those that regulate the potentially autoaggressive subset. The in vivo regulatory interactions between OX-22^{high} and OX-22^{low} subsets that these results demonstrate are discussed and compared with similar regulatory interactions between Th1 and Th2 types of CD4$^+$ T cell clones in vitro (7).

Materials and Methods

Rats. The PVG.RT1c rats used were from the specific pathogen-free (SPF) unit of the Medical Research Council Cellular Immunology Unit (Oxford, UK). Congenitally athymic nude rats (PVG rnu/rnu) were obtained from Harlan Olac (Bicester, UK) or bred in the Cellular Immunology Unit. Nude rats were maintained in filtered cabinets throughout the experiments.

Cells. Thoracic duct lymphocytes (TDL) were obtained by cannulation of the duct (8). Cells 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

[1] *Abbreviations used in this paper:* CSIF, cytokine synthesis inhibitory factor; SPF, specific pathogen-free; TDL, thoracic duct lymphocyte.

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work—i.e., W3/25 (anti-CD4) (9) OX-8 (anti-CD8) (9), OX-12 (anti-rat κ chain) (10), OX-6 (anti-rat MHC class II) (11), OX-39 (anti-rat IL-2R) (12), OX-40 (against a cell surface antigen on CD4+ T cell blasts) (12), OX-22 (anti-rat CD45RB) (1), OX-21 (anti-human C3b inactivator) (13) OX-1 (anti-rat CD45) (14), OX-30 (anti-rat CD45) (15), OX-42 (anti-rat CD18, CD11b) (16), and R73 (anti-rat TCR-α/β) (17)—have all been described in the references given. Biotinylated mAbs were prepared as described (18).

Cell Separation and Flow Cytofluorography. Rat CD4+ T cells were purified from TDL by depletion of B cells, CD8+ T cells, and IL-2R+ cells using a rosetting technique (18). Cell fractionation of the CD4+ population with the OX-22 mAb was carried out on the fluorescence-activated cell sorter (FACS II; Becton Dickinson & Co., Mountain View, CA) as reported previously (3). Two-color flow cytofluorography of TDL from the T cell–replaced nude rats was carried out as described in reference 18 except that fluorescein-conjugated rabbit Fab anti-mouse Ig was used in place of fluorescein-conjugated rabbit $(Fab')_2$ anti-mouse Ig, and the incubation step with mouse serum was omitted. After incubation with streptavidin PE (gift of Dr. S.V. Hunt, Sir William Dunn School of Pathology, Oxford), unconjugated biotin, at a final concentration of 3 μg/ml, was added for 10 min to reduce cell aggregation after centrifugation. Cells were analyzed on a FACSCAN (Becton Dickinson & Co.) or FACS II.

Reconstitution of Nude Rats with T Cell Subpopulations. Nude rats were reconstituted with CD4+ T cell subpopulations as previously described (6). In some cases animals were immunized with 100 μg CFA in the hind footpads.

Immunohistochemistry. Tissues were removed from nude rats 6–10 wk after T cell reconstitution and frozen. 5-μm cryostat sections were cut and stored dry at 4°C. Staining of cells was performed by a peroxidase technique as detailed in reference 19. Briefly, the sections were fixed in ethanol, incubated with mAb, washed, and incubated with a peroxidase-labeled rabbit anti-mouse Ig (Dakopatts Ltd., Copenhagen, Denmark). Positively stained cells were identified with 3,3' diaminobenzidine HCl. The slides were lightly counterstained with Harris' haematoxylin.

Antithyroglobulin ELISA. Flexible flat-bottomed plates (Flow Laboratories, Ayrshire, UK) were coated with 100 μl of a 2 μg/ml solution of rat thyroglobulin overnight at 4°C. Plates were washed three times with PBS containing 0.05% Tween 20 (PBS/Tween) and 100 μl of a 1:50 dilution of rat serum in PBS/Tween added to each well in triplicate. After a 1-h incubation at 20°C, plates were washed and the assay was developed by addition of 100 μl of a 1:1,000 dilution of alkaline phosphatase–conjugated rabbit anti-rat IgG (Sigma Chemical Co., Poole, UK) in PBS/Tween followed by 50 μl of 5 mg/ml *p*-nitrophenyl phosphate (Sigma Chemical Co.) in 10% diethanolamine buffer. After 20 min absorbance at 405 nm was recorded. Positive sera were identified by reference to a panel of normal PVG sera.

Results

Wasting Disease in Nude Rats Given the OX-22^{high} Subset of CD4+ T Cells. Previous studies from this laboratory have shown that both the OX-22^{high} and OX-22^{low} subsets of CD4+ T cells, isolated from the TDL of normal PVG rats, can substantially reconstitute the peripheral CD4+ T cell pool when injected into syngeneic athymic nude rats (6). Despite the similar proliferative capacity of these two subsets, the OX-22 phenotype of the CD4+ T cell inoculum had a profound effect on the subsequent welfare of the recipient animals. Nude rats given the OX-22^{high} fraction of CD4+ T cells developed a lethal wasting disease, with an onset 6–8 wk after T cell reconstitution (Table 1). 10 wk after T cell reconstitution 7 out of 16 animals in this group had died (animals that had lost weight and were unwell were killed), and the remaining animals weighed an average of 89% of their weight at 8 wk. Indeed, animals given this population of CD4+ T cells did less well ($p < 0.01$) than nude rats not given T cells at all (Table 1). In contrast, animals given OX-22^{low} CD4+ T cells continued to thrive throughout the experiment. All of the latter animals showed weight gains (Table 1) and did significantly better than recipients of OX-22^{high} cells ($p < 0.001$). Similarly, animals given unseparated CD4+

Table 1. *Wasting Disease in Nude Rats Give OX-22^{high} CD4+ T Cells*

Phenotype of T cells injected	Survival >8 wk	Change in body weight*	
		Nonimmunized animals	CFA-immunized animals
		% (n)	% (n)
1–5 × 10^6 OX-22^{high} CD4+ T cells	9/16	88 (7)	89.9 (2)
1–5 × 10^6 OX-22^{low} CD4+ T cells	10/10	103.3 (8)	106.5 (2)
1–5 × 10^6 CD4+ T cells	12/12	102.7 (10)	91.8 (2)
Unreconstituted nude rat	8/10	102 (7)	103 (1)

OX-22^{high}-reconstituted nude rats survived less well than unreconstituted animals ($p < 0.01$) or animals given unseparated CD4+ T cells or the OX-22^{low} subset ($p < 0.001$).

* Percentage change in body weight is calculated as the cumulative weight of animals in each group at 10 wk after T cell reconstitution expressed as a percentage of the value obtained at 8 wk. Numbers in parentheses represent the number of animals in each group. Statistical analysis was performed using the Wilcoxon-rank sum test on pooled data from CFA- and non-CFA-immunized animals in each group. CD4+- and OX-22^{low}-reconstituted rats ($p < 0.001$) and unreconstituted nude rats ($p < 0.01$) survived better than animals given the OX-22^{high} subset of CD4+ T cells. There was no significant difference between OX-22^{low} and CD4+ T cell–reconstituted animals.

T cells (60–70% of which are OX-22^{high}) also remained in better health than animals given OX-22^{high} cells alone ($p < 0.001$; Table 1). However, some wasting was evident among animals given unseparated CD4$^+$ T cells, particularly when the animals were immunized at the time of T cell reconstitution with CFA (Table 1). CFA did not have any effect on the incidence or severity of wasting disease in animals in the other groups and this finding will be discussed later.

Immunopathology in T Cell–restored Nude Rats. The wasting disease was associated with severe pathological changes in a number of tissues. Immunohistochemical analysis of tissue sections from the T cell–restored nude rats taken after 6–12 wk revealed extensive mononuclear cell infiltration in the lungs, liver, thyroids, stomach, and pancreas of some animals, with the highest incidence of disease among recipients of the OX-22^{high} subset of T cells. The data are summarized in Table 2.

Diffuse lung pathology was evident in 9 of 10 rats reconstituted with OX-22^{high} CD4$^+$ T cells (Table 2). In these animals the normal lung architecture (shown in Fig. 1 *A*) was completely obscured by infiltrating cells. Immunohistochemical analysis of frozen sections with anti-CD45 mAbs revealed extensive leukocytic infiltrates, composed predominantly of large activated macrophages (Fig. 1 *B*), which expressed the OX-42 antigen (MAC-1, CD11b/CD18) (data not shown). Large numbers of T cells were also present particularly in the perivascular areas (Fig. 1 *D*), and class II expression was detected on the bronchial mucosa in four of four animals given OX-22^{high} CD4$^+$ T cells (data not shown). Lung sections from all (8/8) of the nude rats reconstituted with OX-22^{low} CD4$^+$ T cells were normal (Table 2). Some lung pathology was present in recipients of unseparated CD4$^+$ T cells; 3 of 10 had diffuse infiltration as described above and a further 2 of 10 had focal infiltrates (Table 2). In lungs graded as having focal pathology, areas of normal lung tissue were present as well as some areas with higher than normal numbers of CD45 staining cells, the majority of which were activated macrophages. There were few infiltrating T cells. Focal lung infiltration was common in unreconstituted nude rats (5/6) (Table 2).

Severe liver pathology was a consistent finding among animals reconstituted with OX-22^{high} CD4$^+$ T cells (Table 2). All of the animals examined in this group had extensive leukocytic infiltrates within the periportal connective tissue and piecemeal necrosis. A typical section stained with anti-CD45 mAbs is shown in Fig. 1 *F*. The incidence of diffuse liver pathology was greatly reduced in the recipients of unseparated CD4$^+$ T cells (1/9). The majority of animals in this group (7/9), like those given the OX-22^{low} subset of CD4$^+$ T cells (8/8) showed no liver disease at all (Table 2). Fig. 1 *E* shows an example of anti-CD45 staining on a liver section from an OX-22^{low} reconstituted nude rat. Livers from unreconstituted nude rats were also normal (Table 2).

Lymphocytic thyroiditis was detected in 11 of 23 T cell–restored nude rats and 9 of 26 developed antithyroglobulin antibodies. Unreconstituted nude rats did not have thyroiditis or antithyroglobulin antibodies (Table 2). Interstitial leukocytic infiltrates, detected by staining thyroid sections with anti-CD45 mAbs, were classified as diffuse (Fig. 1 *H*) or focal (Fig. 1 *G*). In animals with thyroiditis, a large proportion of the infiltrating cells stained with mAb OX-42, indicating they were macrophages. Smaller numbers of T cells were also present (data not shown).

The occurrence of thyroiditis depended upon the OX-22 phenotype of the CD4$^+$ T cell inoculum. Seven of nine animals tested in the OX-22^{high} reconstituted group had focal thyroiditis. In contrast, only one of six OX-22^{low} reconstituted animals had an infiltrate: this was of the diffuse type. Of eight animals given unseparated CD4$^+$ T cells, one

Table 2. *Immunopathology in T Cell–restored Nude Rats*

		Thyroids				Liver			Lung			Stomach		Pancreas	
Phenotype of T cells injected	Immunization	0*	1	2	Abs‡	0	1	2	0	1	2	0	2	0	1
1–5 × 10^6 CD4$^+$	0	5	2	1	4/9	6	0	1	5	2	2	2	0	3	0
	CFA	ND	ND	ND	0/2	1	1	0	0	0	1	0	1	0	2
1–5 × 10^6 OX-22^{high} CD4$^+$	0	1	6	0	2/5	0	0	4	1	0	6	1	1	1	1
	CFA	1	1	0	2/3	0	0	3	0	0	3	1	2	0	3
1–5 × 10^6 OX-22^{low} CD4$^+$	0	3	0	1	1/5	5	0	0	6	0	0	2	0	4	0
	CFA	2	0	0	0/2	3	0	0	2	0	0	3	0	2	0
Unreconstituted nude rat	0	3	0	0	0/6	2	0	0	1	4	0	ND	ND	ND	ND
	CFA	ND	ND	ND	ND	1	0	0	0	1	0	ND	ND	1	0

Tissue sections from nude rats reconstituted 6–10 wk previously with CD4$^+$ T cell subsets were examined after immunohistochemical staining as described in Fig. 1.

* Pathology was graded as follows: 0, no pathology; 1, focal infiltration; 2, diffuse infiltration.

‡ Number of animals that had positive antithyroglobulin antibodies in the serum. Data from animals immunized at the time of T cell reconstitution with CFA are presented separately from animals not immunized.

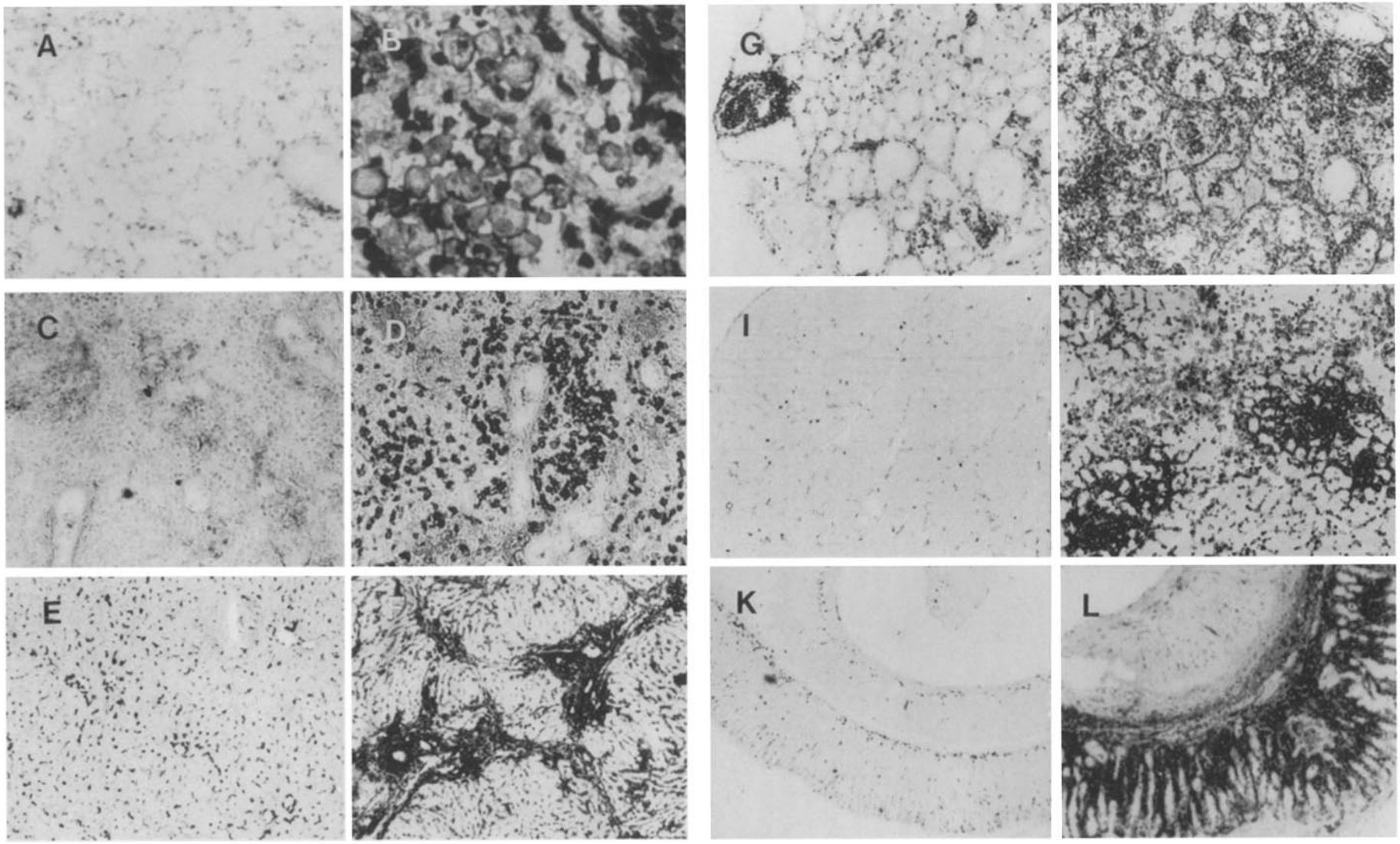

Figure 1. (*A*) Lung tissue from a normal PVG rat stained with anti-CD45 mAbs (OX-1 and OX-30) (×50). (*B*) high power magnification (×400) of lung tissue from an OX-22^{high} CD4$^+$ T cell–restored nude rat stained with OX-1 and OX-30 mAbs, showing a number of large activated macrophages. (*C* and *D*) Serial sections of lung tissue from an OX-22^{high} CD4$^+$ T cell–restored nude rat stained with the negative control mAb OX-21 (*C*) or R73, an anti-rat TCR-α/β mAb (*D*) (×130). (*E* and *F*) Liver sections from an OX-22^{low} (*E*) or OX-22^{high} (*F*) CD4$^+$ T cell–restored nude rat stained with OX-1 and OX-30 mAbs (×64). (*G* and *H*) Thyroid sections from CD4$^+$ T cell–restored nude rats stained with OX-1 and OX-30 mAbs showing focal (*G*) and diffuse (*H*) thyroiditis (×50). (*I* and *J*) Pancreas sections stained with OX-1 and OX-30 mAbs (×50), from OX-22^{low} and OX-22^{high} CD4$^+$ T cell–restored nude rats, respectively, that had been immunized with CFA at the time of T cell reconstitution. (*K* and *L*) Stomach sections stained with OX-1 and OX-30 mAbs: (*K*) from a normal PVG rat (×23); (*L*) from a CD4$^+$ T cell–restored nude rat immunized at the time of T cell reconstitution with CFA (×33).

developed diffuse infiltration and two had focal thyroiditis (Table 2).

Focal pancreatitis was evident in four of five OX-22^{high} CD4$^+$ T cell–reconstituted animals. A representative example is shown in Fig. 1 *J*. CD45$^+$ cells were randomly scattered throughout the section with the endocrine tissue remaining intact. A pancreas from an OX-22^{low} CD4$^+$ T cell–restored nude rat stained with anti-CD45 mAbs is shown in Fig. 1 *I* for comparison. It is notable that in contrast to the incidence of other pathology, pancreatitis appeared to be increased in animals that were immunized with CFA at the time of T cell reconstitution. This increase occurred in recipients of OX-22^{high} or unseparated CD4$^+$ T cells (Table 2). In the latter group, two of two CFA-immunized animals developed focal pancreatitis compared with zero of three among animals that were not immunized with CFA. However, in view of the fact that only a few animals in each group have been immunized with CFA, further experiments will be required to confirm this association. Pancreatitis was completely absent from animals reconstituted with the OX-22^{low} CD4$^+$ T cell subset (0/6) (Fig. 1 *I*).

Evidence of gastritis in the T cell–restored nude rats was also obtained. Three of five OX-22^{high} reconstituted animals and one of three unseparated CD4$^+$ T cell–reconstituted animals had this lesion which was characterized by a severe leukocytic infiltrate (Fig. 1 *L*) and the induction of class II MHC antigen expression on the mucosal epithelium (data not shown). Stomach sections from OX-22^{low} CD4$^+$ T cell–reconstituted nude rats were indistinguishable from those obtained from normal euthymic rats (Fig. 1 *K*). The one CD4$^+$ T cell–reconstituted animal that developed gastritis had also been previously immunized with CFA (Table 2).

T Cell Subsets in T Cell–restored Nude Rats. Analysis of TDL 8–10 wk after T cell reconstitution showed that animals given the OX-22^{high} CD4$^+$ subpopulation had reconstituted their T cell pool to near normal levels (~47% CD4$^+$ T cells), whereas OX-22^{low} and unseparated CD4$^+$ T cell–reconstituted rats contained fourfold and twofold fewer CD4$^+$ T cells, respectively (Table 3). The CD4$^+$ T cell population in all of the T cell–restored animals had a higher percentage of cells expressing the activation antigens IL-2R and OX-40 (a CD4$^+$ T cell blast antigen [12, 20]) than found in normal PVG rats (Table 3). There were also differences between the groups that received different CD4$^+$ T cell subpopulations. Animals reconstituted with the OX-22^{high} fraction of CD4$^+$ T cells contained the highest frequency of activated CD4$^+$ T cells (65% OX-40$^+$). In total numbers, this figure is eightfold higher than the number of OX-40$^+$ CD4$^+$ T cells found in animals reconstituted with the OX-22^{low} subset of CD4$^+$T cells and fourfold higher than in animals given unseparated CD4$^+$ T cells (Table 3). The finding that the majority of CD4$^+$ T cells in the animals originally given OX-22^{high} CD4$^+$ T cells were OX-22^{low} (Table 3) is consistent with these cells being activated, as the expression of the OX-22 antigen is known to be lost on T cell activation in vitro (21).

Discussion

The results of this study demonstrate further the functional heterogeneity of the peripheral CD4$^+$ T cell populations defined by the level of expression of the OX-22 antigen. Transfer of small numbers of OX-22^{low} CD4$^+$ T cells into nude rat recipients was protective, and the animals continued to thrive throughout the experiment. In contrast, animals injected with the OX-22^{high} CD4$^+$ T cell subset developed a lethal wasting disease characterized by severe liver and lung pathology and a high incidence of thyroiditis and antithy-

Table 3. *Activation Markers on CD4$^+$ T Cells in T Cell-Restored Nude Rats*

Phenotype of T cells injected	TDL output in 18 h	Percent positive cells in TDL			
		CD4$^+$	OX-22^{high} CD4$^+$	OX-39$^+$ CD4$^+$	OX-40$^+$ CD4$^+$
	$\times 10^{-8}$				
1–5 × 10^6 OX-22^{high} CD4$^+$ T cells	1.47 ± 0.5*	47 ± 7	2.0 ± 1 (4.3)	17 ± 7 (36)	30 ± 9 (65)‡
1–5 × 10^6 OX-22^{low} CD4$^+$ T cells	1.8 ± 0.4	11 ± 2	2.1 ± 0.6 (19)	3.8 ± 0.9 (33)	4 ± 1.3 (35)‡
1–5 × 10^6 CD4$^+$ T cells	1.9 ± 0.6	17 ± 4	2.9 ± 0.2 (17)	4.5 ± 1 (26)	5.4 ± 4 (32)‡
Unreconstituted nude rat	0.35 ± 2	5 ± 2	1.5 ± 0.6 (32)	1.8 ± 0.8 (38)	1.8 ± 0.9 (39)§
Normal PVG	4.6 ± 1	36 ± 5	23 ± 4 (64)	1.6 ± 1.5 (4.6)	2.5 ± 0.35 (7)

Athymic nude rats were reconstituted with CD4$^+$ T cell populations as outlined in Materials and Methods. 8–10 wk later the thoracic duct of the recipients was cannulated and the TDL collected overnight were phenotyped by two-color immunofluorescence on a FACS or FASCAN, using biotinylated W3/25 mAb in combination with other mAbs.

* Data represent the mean with SE for five animals in each group, except for unreconstituted nude rats, where there were four animals in the group. Numbers in parentheses are data expressed as a percentage of the CD4$^+$ T cell population.

‡ $n = 4$.

§ $n = 3$.

roglobulin autoantibodies. Pancreatitis and gastritis were also noted, albeit with lower frequency. The severe leukocytic infiltration of a number of organs and the presence of anti-thyroglobulin autoantibodies suggests an immune-mediated pathogenesis in recipients of the OX-22^{high} subpopulation that is dependent on the T cells transferred as unreconstituted nude rats survived significantly better than animals given OX-22^{high} CD4$^+$ T cells and did not develop autoantibodies or multiorgan inflammation.

The finding that animals given unseparated CD4$^+$ T cells survived as well as animals given the OX-22^{low} subset alone and significantly better than animals given the OX-22^{high} CD4$^+$ T cell subset suggests that the OX-22^{low} subset may in some way regulate the autoaggresive cells contained within the OX-22^{high} subset. The functional heterogeneity within the CD4$^+$ T cell population, illustrated by these data, is in accord with a number of previous studies. On one hand, CD4$^+$ T cells have been shown to be essential mediators in many models of autoimmunity such as multiple-organ localized disease in recipients of rat thymic grafts (22); syngeneic graft-vs.-host disease (23); experimental allergic encephalomyelitis (24); and experimental allergic thyroiditis (25). However, while the presence of some T cells is essential for the induction of autoimmune disease, there is a wide body of literature suggesting that susceptibility to autoimmunity is favored by a relative deficiency in T cells. PVG rats that were thymectomized and sublethally irradiated spontaneously developed thyroiditis with accompanying anti-thyroglobulin antibodies (26) and diabetes (Penhale, W. J., Murdoch University, Perth, W. Australia, personal communication). Similarly, thyroiditis is induced in B mice reconstituted with Lyt-1^{low} T cells (27), and a variety of organ-specific autoimmune diseases such as prostatitis and gastritis have been reported in nude mice reconstituted with T cells (28). It has been suggested that in such lymphopaenic animals, a regulatory population of T cells is missing. This suggestion is based on the finding that in a number of autoimmune diseases the cotransfer of large numbers of CD4$^+$ T cells can prevent disease (27, 29). There are also a number of other examples of CD4$^+$ T cell–mediated suppression in the literature (30, 31), although in none of these has the CD45 phenotype been examined. The present data suggest that the autoaggressive subpopulation of CD4$^+$ T cells can be phenotypically distinguished from the regulatory population based on the level of expression of different isoforms of the CD45 antigen.

Animals injected with the OX-22^{high} subset consistently contained fourfold greater numbers of CD4$^+$ T cells in their TDL than recipients of the OX-22^{low} subpopulation, the majority of which were activated. The stimulus to which these cells are responding is unknown at present: the expansion may be driven entirely in response to environmental antigens; alternatively, the cells may be responding to autoantigens in a manner similar to the in vitro autologous MLR (32). Although numbers are small, the finding that CD4$^+$ T cell–reconstituted nude rats immunized with CFA developed the wasting disease, as well as gastritis and pancreatitis, which was not present in nonimmunized recipients of CD4$^+$ T cells, suggests a role for exogenous antigens. However, if CFA acts to promote disease induction, it seems to require cells contained in the OX-22^{high} subpopulation as there was no difference between CFA immunized and nonimmunized recipients of OX-22^{low} CD4$^+$ T cells or unreconstituted nude rats. CFA is a complex antigenic mixture and further experiments are required to define the component of CFA that may promote disease. Further evidence for a role for environmental antigens in development of autoimmune disease comes from a recent report in which susceptibility of SPF rats to induction of experimental allergic thyroiditis by thymectomy and irradiation was augmented by transfer of intestinal material from conventionally reared animals, implicating the intestinal microflora as a factor in disease induction (33). Uncertainties regarding the role of environmental antigens in autoimmunity are not limited to the present study but are a recurrent problem in this field.

The mechanism by which the OX-22^{low} population regulates the otherwise lethal activity of the OX-22^{high} population is at present unclear. As previously described, OX-22^{high} and OX-22^{low} CD4$^+$ T cells mediate distinct immunological functions. OX-22^{high} CD4$^+$ T cells are the most potent producers of IL-2 and IFN-γ (4), whereas the OX-22^{low} subpopulation provides B cell help (3) and produces the majority of IL-4 (5). Based on lymphokine repertoire, the OX-22 subsets of peripheral CD4$^+$ T cells resemble the subdivision of mouse T cell clones into IL-2 and IFN-γ producers (Th1) or IL-4 producers (Th2). A number of recent studies have demonstrated important regulatory interactions between Th1 and Th2 CD4$^+$ T cell clones: IFN-γ produced by the Th1 clones inhibits the proliferation of Th2 clones (34), and the Th2 product cytokine synthesis inhibitory factor (CSIF) inhibits lymphokine secretion by Th1 clones (35). IL-4 has also been shown to suppress the production of IFN-γ by human PBL (36, 37) and to inhibit the cell-mediated response to *Leishmania major* in mice (38). In view of these data, it seems possible that cells contained within the OX-22^{low} population regulate the expansion of cells in the OX-22^{high} population by the secretion of inhibitory lymphokines such as IL-4. In support of this idea, preliminary data suggest that LN cells from animals reconstituted with a mixture of OX-22^{high} and OX-22^{low} CD4$^+$ T cells spontaneously produce high levels of IL-4, but those given the OX-22^{high} subset alone do not. In this regard, it would be of value to determine which CD4$^+$ T cell population produces CSIF and whether this plays any role in the regulation of the wasting disease.

The majority of CD4$^+$ T cells in the TDL of both OX-22^{high} and OX-22^{low} CD4$^+$ T cell–reconstituted recipients are OX-22^{low} 8–10 wk after T cell reconstitution. This finding suggests that in this system at least, the functional phenotype of the OX-22^{high} population is stable despite loss of the cell surface marker. However, the finding that the majority of CD4$^+$ T cells in the OX-22^{high} reconstituted rats were activated make it impossible to determine whether these cells generate resting memory cells with the OX-22^{high} phenotype. In a previous report, it was established that the OX-22^{low} CD4$^+$ T cells reactive in assays of secondary B cell help derive from precursors with the OX-22^{high} phenotype (6). If the OX-22^{high} population contains the precursors of

cells that can provide B cell helper function (Th2-like cells), then the question arises as to why a population of regulatory cells (also presumably Th2 cells) are not also generated from the expansion of OX-22^{high} precursor cells in the nude rats in sufficient number to prevent the wasting disease. There are several possible reasons for this. For example, (*a*) the environment in the nude rat may not allow the generation of the regulatory cells from naive precursors, or the rate at which they are generated is too slow. (*b*) The regulatory cells may be distinct from those that help B cells and do not derive from OX-22^{high} precursors. The OX-22^{low} CD4$^+$ T cell population is known to be phenotypically heterogeneous (39) with respect to expression of the Thy-1 and RT6 antigens (40) but the full significance of this division is unknown at present. (*c*) The antigen specificities in the two CD4$^+$ subsets may be different.

Data presented in this paper raise a number of questions important to the understanding of immunoregulation and illustrate the profound consequences of the injection of one CD4$^+$ T cell subpopulation in the absence of its physiological counterpart. The data provide further support for the concept of functional specialization within the CD4$^+$ T cell population previously proposed (7, 39). We have recently reported that the human memory CD4$^+$ T cell population can be subdivided based on the level of expression of the CD45RB epitope and have stressed that the complex mechanism for generating the different isoforms of CD45 has been preserved in ontogeny (4). In view of the regulatory interactions between the CD4$^+$ T cell subsets identified by reactivity with OX-22 (CD45RB) described herein and the severe pathology that appears when these interactions are abrogated, it may be reasonably suggested that some autoimmune diseases in humans may develop as a consequence of a malfunction of this mechanism. It is notable in this context that the pathological changes in the liver, stomach, thyroid, and pancreas reported here are not dissimilar to those associated with organ-specific inflammatory disease in humans.

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