SPECIES HETEROGENEITY IN MACROPHAGE EXPRESSION OF THE CD4 ANTIGEN

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The CD4 antigen is a plasma membrane glycoprotein of ~55 kD that is expressed on most thymocytes and on Th cells in all mammalian species examined, including human, rat, mouse, sheep, and pig (1). The antigen is also present on rat macrophages (Mφ) and human monocytes in a similar molecular form (2, 3). Studies with mAbs (4) have demonstrated that CD4 on Th cells is an important accessory molecule involved in recognition of antigen plus MHC class II molecules during immune responses. In humans, a more recently defined property of the CD4 antigen is to act as the receptor for the AIDS virus (human immunodeficiency virus or HIV) (5). Although nothing is known concerning the the regulation and functional significance of CD4 expression on Mφ, its presence is likely to be at least one reason why these cells become infected by HIV (6, 7). Here we demonstrate that, unlike human and rat Mφ, mouse Mφ do not express the CD4 (L3T4) antigen. This species disparity indicates, firstly, that CD4 may not be essential for Mφ function, and secondly, that Mφ regulate CD4 expression differently from Th cells.

Materials and Methods

Animals. Specific pathogen-free C57BL/6 mice and AO rats were bred at the Sir William Dunn School of Pathology and both sexes were used between 6 and 12 wk of age.

Antibodies. The following rat mAbs to mouse CD4 (L3T4) were obtained as shown and used as tissue culture supernatants at saturation: H129.19 (IgG2a) (8), RL 172.4 (IgM) (9), and GK1.5 (IgG2b) (10), provided by Dr. H. R. MacDonald, Ludwig Institute for Cancer Research, Lausanne, Switzerland; and YTS 191.1 (IgG2b) and YTA.3 (IgG2b) (11), provided by Dr. S. P. Cobbold, Department of Pathology, Cambridge University, Cambridge, United Kingdom. The noncompeting mouse anti–rat CD4 (W3/25) mAbs, W3/25 (IgGI), and MRC OX-35 (IgG2a) (2), were from the MRC Cellular Immunology Research Unit, University of Oxford, Oxford, United Kingdom. Other mAbs used were MRC OX-21 (IgG1), a mouse mAb to human C3b inactivator (2), and F4/80 (IgG2b), a rat mAb specific for mature mouse Mφ (12).

Labeling of Cells with Antibodies. Mouse and rat resident peritoneal Mφ were purified by adherence for 1 h to bacteriologic petri dishes followed by extensive washing. The adherent cells (>90% Mφ by morphology) were detached by a 15-min incubation in calcium- and magnesium-free PBS at 37°C followed by gentle pipetting. Thymocytes

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were obtained by teasing thymuses in PBS containing 0.1% BSA and 10 mM sodium azide (PBA). Thymocytes and MΦ were labeled at 4°C with first antibody diluted in PBA followed either by FITC-conjugated OX12 F(ab')2 (mouse anti-rat κ chain, used with mouse cells) or FITC-rabbit anti-mouse F(ab')2 (with rat cells). Fluorescence histograms were obtained on a Becton Dickinson & Co. (Mountain View, CA) FACS II with 10^6 cells analyzed for each profile and with cell number shown on a linear scale.

**Northern Blot Hybridization.** Mouse and rat thioglycollate-elicted peritoneal MΦ (12) were purified (>95%) by adherence for 1 h to tissue culture–treated petri dishes followed by extensive washing to remove lymphocytes. Human monocytes were purified similarly and cultured for 12 d in RPMI 1640 containing 10% autologous serum. RNAs were isolated as described (13) and where indicated, poly(A)+ RNA was selected by oligo(dT) cellulose chromatography. Rat and mouse RNA samples were resolved on a 1.2% agarose-formaldehyde gel, transferred to Gene Screen (New England Nuclear, Boston, MA) and hybridized with both nick-translated rat CD4 cDNA (1) and human lysozyme cDNA (L. P. Chung, unpublished). The filter was washed with 2x SSC at 65°C and exposed to autoradiographic film for 5 d. The human sample was resolved on a 1.0% agarose-formaldehyde gel, transferred to Gene Screen, and hybridized with human lysozyme cDNA or human CD4 cDNA (14), kindly provided by Dr. P. J. Maddon, Columbia University, New York. The filter was washed with 3x SSC at 65°C and exposed for 1 (lysozyme) or 7 d (CD4).

**Immunoperoxidase Analysis.** 10-μM tissue cryostat sections were fixed with acetone and stained using an avidin-biotin immunoperoxidase detection system (Vectastain; Seralab, Crawley Down, United Kingdom). Preparations were counterstained with Mayer’s hemalum and photographed using a dark blue filter.

**Results and Discussion**

**FACS Analysis.** To investigate the expression of CD4 (L3T4) on mouse peritoneal MΦ we first tested by FACS analysis the binding of four competing rat anti-mouse CD4 (L3T4) mAbs of different isotypes. Both freshly harvested and adherence-purified resident or inflammatory peritoneal MΦ were studied, but in six independent experiments we were unable to demonstrate labeling of MΦ from either C57BL/6 or BALB/c mice. Mouse thymocytes were clearly labeled by each antibody in each experiment. In a representative experiment, shown in Fig. 1, there was no binding of H129.19 (8) to purified resident peritoneal MΦ above the background levels using FITC-OX12 F(ab')2 alone (Fig. 1a), while mouse thymocytes showed clear labeling (Fig. 1c). The failure of mouse MΦ to label with antibodies to CD4 (L3T4) was not due to poor viability since the majority were strongly labeled with the Mo-specific mAb F4/80 (12) (Fig. 1a), and >85% excluded trypan blue after each experiment. In addition, rat (AO) resident peritoneal MΦ purified simultaneously by the same method were strongly labeled with either OX35 or W3/25, two noncompeting mouse mAbs to rat CD4 (2) (Fig. 1b and d).

**Northern Blot Hybridization.** Direct evidence that mouse peritoneal MΦ do not express the CD4 (L3T4) antigen was provided by Northern blot analysis using a rat CD4 cDNA probe (1) (Fig. 2). As an internal control for the presence of Mφ RNA, we also screened the filter with a cDNA probe to human lysozyme mRNA. It can be seen in Fig. 2, lane 4, that with mouse thymocytes the expected 3.7-kb CD4 (L3T4) mRNA was readily detected by crosshybridization of the rat probe with <20 μg total mouse thymocyte RNA. In contrast, no CD4 (L3T4) mRNA could be detected in mouse MΦ (Fig. 2, lanes 5–8), even with up to 10 μg purified mRNA [poly(A)+] and with prolonged exposure (3 wk). In addition, CD4 (L3T4) mRNA was not induced by 3-d in vitro cultivation of mouse
**Figure 1.** FACS analysis to investigate CD4 expression on mouse \( \Phi \) and thymocytes in comparison with rat \( \Phi \) and thymocytes. (a) Purified mouse resident peritoneal \( \Phi \) showing undetectable labeling with H129.19 (anti-mouse CD4 (L3T4)) compared with background labeling with fluoresceinated second antibody alone (FITC). Labeling with F4/80 shown as a positive control. (b) Purified rat resident peritoneal \( \Phi \) labeled with OX35 (anti-rat CD4 (W3/25)) compared with OX21 used as negative control. (c) Mouse thymocytes labeled with H129.19 compared with F4/80 used as a negative control. (d) Rat thymocytes labeled with OX35 compared with OX21. Rat anti-mouse CD4 (L3T4) mAbs tested in addition were GK1.5, YTS 191.1, and RL 172.4. The noncompeting mAb to mouse CD4 (L3T4), YTA.3, nonspecifically labeled ~50% of \( \Phi \) via binding to Fc receptors (data not shown).

**Figure 2.** Northern blot analysis of RNAs isolated from thymocytes and purified \( \Phi \), probed with either human (lane 1) or rat (lanes 2–8) CD4 cDNA and human lysozyme cDNA. (1) 20 \( \mu \)g total RNA from purified human monocytes cultured for 12 d and probed with CD4 mRNA (top panel) or lysozyme mRNA (bottom panel); (2) 20 \( \mu \)g total RNA from rat exudate peritoneal \( \Phi \); (3) 10 \( \mu \)g total RNA from rat thymocytes; (4) 18 \( \mu \)g total RNA from mouse thymocytes; (5 and 6) 10 \( \mu \)g and 2 \( \mu \)g poly(A)\(^+\) RNA from mouse exudate peritoneal \( \Phi \) respectively; (7) 20 \( \mu \)g total RNA from mouse exudate peritoneal \( \Phi \); (8) 20 \( \mu \)g total RNA from mouse exudate \( \Phi \) after culture for 3 d.
Immunocytochemical analysis of mouse spleen and liver demonstrating absence of CD4 (L3T4) on tissue Mφ. (a) Staining of T cells but not red pulp (RP) Mφ for CD4 (L3T4) in spleen; (b) lack of staining of Kupffer cells for CD4 (L3T4) in liver; (c) staining of red pulp (RP) Mφ with F4/80 in serial section of spleen; (d) staining of Kupffer cells with F4/80 in serial section of liver. The asterisks in a and c overlie a splenic arteriole that is common to both sections. Scale bars indicate 20 μM.

peritoneal Mφ (Fig. 2, lane 8) and could not be detected in total RNA samples from the murine Mφ-like cell line J774.2 (data not shown). There was also no evidence of hybridization to a 2.7-kb CD4 mRNA species recently demonstrated in poly(A)⁺ RNA isolated from human and mouse brain (5, 15). Since human (but not mouse) brain also expresses the normal sized CD4 mRNA (5), our results lend support to the suggestion that the 2.7-kb CD4 mRNA is derived from cells other than microglia (brain Mφ) (5). The presence of intact mouse Mφ mRNA in each lane was confirmed by the strong signals obtained by hybridization of the lysozyme probe to 1.5-kb mouse lysozyme mRNA. The lower molecular weight bands (<1.5 kb) observed with some mouse Mφ samples (Fig. 2, lanes 5 and 7) were due to hybridization to the lysozyme probe. In contrast to the mouse, human and rat Mφ clearly expressed CD4 mRNA in addition to lysozyme mRNA (Fig. 2, lanes 1 and 2).

Immunocytochemistry. To determine whether other mouse tissue Mφ populations failed to express the CD4 (L3T4) antigen we investigated its expression on spleen and liver Mφ by using a sensitive avidin-biotin immunoperoxidase detection system and mixtures of the noncompeting mAbs, YTS 191.1 and YTA.3 (11) (Fig. 3). In mouse spleen there was clear labeling of T cells, but Mφ in the marginal zone or red pulp were not stained (Fig. 3 a). With mouse liver there was no labeling of any cell population for CD4 (L3T4) antigen (Fig. 3 b). To control for the presence of structurally intact Mφ in both tissues, serial sections were stained with mAb F4/80. This revealed intense labeling of Mφ in the red
pulp of the spleen (Fig. 3c), while in liver, Kupffer cells were clearly evident (Fig. 3d). These results are, again, in stark contrast to the rat in which labeling of splenic Mφ and Kupffer cells could easily be detected by conventional two-layer immunoperoxidase techniques (not shown).

In view of the 2.7-kb CD4 (L3T4) mRNA reported in mouse brain (5, 15), we also examined sections of this tissue. However, in contrast to the human and rat (reference 16, and Perry, V. H., personal communication), we have so far not observed specific staining for CD4 (L3T4) in mouse brain, either on microglia, choroid plexus Mφ, or other cell populations. Finally, CD4 (L3T4) expression was not detectable on activated peritoneal Mφ obtained from BCG-infected mice (not shown).

These results demonstrate that expression of CD4 on Th cells and Mφ can be uncoupled. This implies that each cell type regulates the expression of CD4 by differing mechanisms. If this is the case, mouse Mφ may be suppressed or lack one or more of the regulatory components required. As yet, the significance of CD4 on Mφ is unknown but if the molecule performs essential functions in species such as the rat and human, then the mouse must have evolved different mechanisms to carry these out. Alternatively, CD4 may be nonessential or even without a function on Mφ. In humans, the only known 'function' of CD4 on monocytes is to act as a receptor for HIV, resulting in a low-grade but persistent infection of these cells in vitro (6, 7). Clearly, the species heterogeneity in Mφ expression of the CD4 antigen should be taken into account when assessing the use of animal models of HIV infection, since it is possible that the relative number of CD4 molecules on tissue Mφ is an important determinant of their innate susceptibility to infection by HIV in vivo. In rats, Mφ display similar levels of CD4 to Th cells (Jefferies, W. A., unpublished observations), but whether an analogous situation exists in humans is unknown. An understanding of the mechanisms by which Mφ regulate expression of CD4 is therefore of direct relevance not only to the functional significance of the molecule on these cells, but also with respect to its role as a human Mφ receptor for HIV.

Summary

The CD4 antigen is expressed on T cells of all mammalian species examined and appears to play an important role in the response of T cells to antigen. In humans, the molecule acts as a receptor for the AIDS virus. Previous studies (2, 3) have demonstrated that Mφ in the rat and human also express the CD4 antigen, which is indistinguishable from that on T cells. In this paper we demonstrate by FACS analysis, Northern blot hybridization, and immunoperoxidase labeling that, in striking contrast to the rat and human, mouse Mφ do not express the CD4 (L3T4) antigen. This species heterogeneity indicates that T cells and Mφ regulate CD4 antigen expression independently and that CD4 may not be essential for Mφ function.

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