The Role of CD4 T Cells in the Pathogenesis of Murine AIDS

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LP-BM5, a retroviral isolate, induces a disease featuring retrovirus-induced immunodeficiency, designated murine AIDS (MAIDS). Many of the features of the LP-BM5-induced syndrome are shared with human immunodeficiency virus-induced disease. For example, CD4 T cells are central to the development of MAIDS. In vivo depletion of CD4 T cells before LP-BM5 infection renders genetically susceptible B6 mice MAIDS resistant. Similarly, MAIDS did not develop in B6.nude mice. However, if reconstituted with CD4 T cells, B6.nude mice develop full-blown MAIDS. Our laboratory has shown that the interaction of B and CD4 T cells that is central to MAIDS pathogenesis requires ligation of CD154 on CD4 T cells with CD40 on B cells. However, it is not clear which additional characteristics of the phenotypically and functionally heterogeneous CD4 T-cell compartment are required. Here, in vivo adoptive transfer experiments using B6.nude recipients are employed to compare the pathogenic abilities of CD4 T-cell subsets defined on the basis of cell surface phenotypic or functional differences. Th1 and Th2 CD4 T cells equally supported MAIDS induction. The rare Thy1.2+/CD4 subset that expands upon LP-BM5 infection was not necessary for MAIDS. Interestingly, CD45RBlow CD4 T cells supported significantly less disease than CD45RBhigh CD4 T cells. Because the decreased MAIDS pathogenesis could not be attributed to inhibition by CD45RBhigh CD25+ natural T-regulatory cells, an intrinsic property of the CD45RBlow cells appeared responsible. Similarly, there was no evidence that natural T-regulatory cells played a role in LP-BM5-induced pathogenesis in the context of the intact CD4 T-cell population.

The LP-BM5 retroviral isolate consists of a pathogenic defective murine leukemia virus (BM5def) that requires the replicative-competent ecotropic helper virus BM5eco for its entry into cells and spread in vivo. The ensuing AIDS variety, termed murine AIDS (MAIDS) (25), exhibits many features shared with human immunodeficiency virus (HIV)-induced disease in humans (3, 7, 55). These similar features include early-onset hypergammaglobulinemia (hyper-Ig), splenomegaly, and lymphadenopathy; dependence on CD4 T cells for initiation of disease; loss of CD4 T-cell function; severely depressed T- and B-cell responses; increased susceptibility to infection and death when exposed to normally nonpathogenic microorganisms; and the development of terminal B-cell lymphomas. These MAIDS-associated “opportunistic” lymphomas occur under these immunodeficiency conditions in susceptible C57Bl/6 mice and are generally similar to the lymphoid tumors observed in human AIDS patients.

Interaction between B and CD4 T cells is critical for the pathogenesis of MAIDS; both CD4 T cells and B cells are required for disease induction and progression. Severe combined immunodeficient (SCID) mice (lacking mature T and B cells) do not develop MAIDS (52). The main targets of LP-BM5 retrovirus infection are B cells and, to some extent, macrophages and T cells. However, B-cell infection is not progressive and does not lead to disease in the absence of mature CD4 T cells (8, 23). Indeed, an early hyperactivity of CD4 T cells may contribute to the pathogenesis of MAIDS (39, 60). In vivo depletion of CD4 T cells before LP-BM5 infection rendered susceptible B6 mice resistant to the development of MAIDS. Similarly, congenitally athymic nude B6 mice are resistant to LP-BM5-induced disease (39). However, if reconstituted with CD4 T cells from B6 donor mice, B6.nude recipients develop MAIDS upon LP-BM5 infection (15, 19, 60). In further adoptive transfer experiments it was found that B6.nude recipients, if reconstituted with CD154+/+, but not CD154−/−, CD4 T cells, were converted to disease susceptibility (19). With regard to the cell type interacting with the CD154+ CD4 T cells, B6 CD40−/− mice, which are relatively resistant to LP-BM5-induced MAIDS, became susceptible to LP-BM5-induced disease after reconstitution with highly purified wild-type B cells but not after receiving purified wild-type dendritic cells or a combined CD40+ population composed of dendritic cells and macrophages obtained from B6.SCID mouse donors (19). In a recent follow-up study, we showed that the CD80/CD86 (B7.1/B7.2) costimulatory ligands are not required for LP-BM5 induction of MAIDS (17). These results and other data (36) suggested that CD4 T-cell-expressed CD28 ligation is not required for the initiation of MAIDS or the early commitment to disease progression. Although this classic upregulation of CD80/CD86 after CD40 ligation is thus not necessary, we have used a panel of CD40 Tg mice on the CD40−/− background to confirm that the CD40 signaling is required for MAIDS induction and is differentially mediated by TRAF binding to the TRAF 6 site (versus the TRAF 2/3/5 site) on the CD40 cytoplasmic tail (16). Collectively, these findings provide clear evidence that activated CD4 T-cell CD154 ligation of B-cell CD40 and downstream signaling events are required for MAIDS (16, 19).

Beyond this series of reports, however, surprisingly little is...
known about other CD4 T-cell features required for the induction of MAIDS. Several studies have examined the effects of established MAIDS disease on the CD4 T-cell compartment, i.e., characterization of CD4 T-cell anergy (2, 21, 41, 59), but there are few prospective studies defining the role of these CD4 T-cell features in the induction of MAIDS. The molecular basis for the requirement for CD4 T cells for MAIDS induction is thus incompletely understood. The relevant question is as follows: which phenotypic and/or functional characteristics of CD154^+ CD4 T cells are critical for mediating MAIDS? To address the role of CD4 T cells in MAIDS induction and pathogenesis, we performed a series of in vivo adoptive transfer experiments designed to determine which subpopulation(s) of CD154^+ CD4 T cells, as defined on the basis of relevant cell surface phenotypic and functional differences, are essential for LP-BM5-induced MAIDS. This approach allows direct comparisons in a system where MAIDS induction is dependent on the transferred cells.

**MATERIALS AND METHODS**

Mice. Male B6 mice were purchased from the National Institutes of Health (Bethesda, MD), housed in the Dartmouth Medical School Animal Resource Center, and used when they reached 8 to 10 weeks of age as control mice or spleen cell donors. B6.nude breeder mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were bred to increase group size at the Dartmouth Medical School Animal Resource Center. Equal numbers of B6.nude mice, either three or four in a given experimental group, were reconstituted with various cell preparations (see below) before infection with LP-BM5 virus.

**LP-BM5 virus inoculations.** LP-BM5 virus was prepared in our laboratory as previously described (22). Briefly, 10^9 cells cloned from SC-1 fibroblasts infected with the LP-BM5 virus mixture and originally provided by Janet Hartley and Herbert Morse (NIH/NIAID, Bethesda, MD) were cocultured with uninfected SC-1 cells. Mice were infected intraperitoneally (i.p.) with 1 x 10^7 ecotropic PFU stock virus.

**Splenocyte subpopulation preparations.** Splenocyte suspensions were labeled with various antibody-coupled paramagnetic beads (MACS; Miltenyi Biotec, Auburn, CA) and subjected to column purification according to the manufacturer's protocol or sorted by use of a FACStar Plus instrument with TurboSort (Becton Dickinson Immunocytometry Systems, Mountain View, CA). The purified cellular subsets indicated below were used in reconstitution experiments; in each case, a portion of the recipients of such adoptivetransfer was infected with LP-BM5 48 h after adoptive transfer, and the remaining mice receiving adoptive cell transfer served as uninfected controls.

(i) Generation of CD4 T-cell preparations. Spleen cell suspensions were labeled with anti-CD4 beads, and positive selection yielded cell preparations which were $\geq 98\%$ CD4 and less than 1% B220 and CD8, as detected by flow cytometric analysis. Alternatively, negative selection of CD4 T cells was accomplished by using anti-CD8, -CD19, -CD11b, and -CD11c beads; for the collected flowthrougth, designated as CD4 negatively selected cells, the purity was $\geq 98\%$, CD4 T cells ($1 \times 10^7$ unless otherwise indicated) obtained by either positive or negative selection were adoptively transferred intravenously into B6.nude recipients.

(ii) Generation of CD4 Th1-like and Th2-like cell preparations in vitro. Th1 and Th2 cells were induced from B6 mice as follows (Fig. 1). (27). Either unfractionated purified CD4 T cells or sorted CD62L$^{\text{high}}$ CD44$^{\text{low}}$ naive CD4 T cells from B6 mice spleen cell cultures were cultured in tissue culture plates (precoated with 10 mg/ml anti-CD3 monoclonal antibody [MAb] [clone 145-2C11; BD Pharmingen] and 1 mg/ml anti-CD28 MAb [37B9; BD Pharmingen]) in the presence of either 5 ng/ml interleukin-12 (IL-12; PeproTech) and neutralizing 10 $\mu$g/ml anti-IL-4 MAb (11B11; BD Pharmingen) for Th1 cells or of 5 ng/ml IL-4 (PeproTech) with neutralizing 10 $\mu$g/ml anti-gamma interferon (IFN-γ) MAb (104-18H12; BD Pharmingen) and 10 ng/ml anti-CD25 (IL-2Rα) MAb used in vivo was generated from hybridoma PC61 (29, 42) and purified via elution over a protein G agarose column (Sigma). The antibody immunoglobulin G (IgG) concentration was determined by comparison to commercial rat anti-mouse CD25 MAb (PC61; eBioscience) in flow cytometric analyses employing FITC-labeled goat anti-rat secondary antibody and indirect immunofluorescence as a standard. Purified rat-IgG1 was used as a normal rat IgG control. Then, spleen cells from these anti-CD25 MAb-treated donor mice were processed in a fashion identical to that described above to yield CD45RB$^{\text{high}}$ CD4$^{\text{+}}$ CD45RBlow CD25$^{\text{−}}$ CD4 T cells.

(iii) Depletion of Thy1.2$^+$ CD4 T cells. Unfractionated, purified splenic CD4 T cells from normal B6 donor mice were stained with fluorescein isothiocyanate (FITC) anti-Thy1.2 and phycoerythrin (PE) anti-CD4. Labeled CD4 T cells were sorted by use of a FACStar Plus instrument with TurboSort to isolate the Thy1.2$^+$ CD4 population. The purity of the resulting Thy1.2$^+$ CD4 population was $\geq 98\%$. Either these cells or unfractionated CD4 T cells as a positive control (1 x 10^7) were adoptively transferred into B6.nude recipients.

(iv) Isolation of CD45RB$^{\text{high}}$ naive CD4 T cells and CD45RB$^{\text{low}}$ memory CD4 T cells from normal B6 spleenocytes and adoptive transfer into B6.nude recipients. First, splenic CD4 T cells from normal B6 donor mice were purified by using Miltenyi beads as detailed above. After saving enough CD4 T cells for use as the unfractionated polyclonal CD4 T-cell control, we labeled the rest of the CD4 T cells with FITC anti-CD4 (H129.19) and PE anti-CD45RB (16A) (BD Pharmingen). These labeled cells were sorted at high speed on a FACStar Plus instrument with TurboSort. Based on our and another laboratory's experience (28), the CD45RB$^{\text{high}}$ and CD45RB$^{\text{low}}$ populations were defined as the CD45RB bright-staining 30% and dullest-staining 15%, respectively, of the CD4 cells. Purity of CD45RB$^{\text{high}}$ CD4 and CD45RB$^{\text{low}}$ CD4 populations was $\geq 98\%$. CD4 T cells (0.5 x 10^7 to 1 x 10^7) (either CD45RB$^{\text{high}}$, CD45RB$^{\text{low}}$, or unfractionated CD4 T cells as a positive control) were transferred into each B6.nude recipient.

(v) Depletion of CD45RB$^{\text{low}}$ CD25$^{\text{−}}$ CD4 T-cell regulatory cells. Unfractionated, purified splenic CD4 T cells from normal B6 donor mice and CD4 T cells sorted into CD45RB$^{\text{high}}$ and CD45RB$^{\text{low}}$ subsets were obtained as described immediately above, except that the CD45RB$^{\text{low}}$ CD4 T cells were separated into CD45RB$^{\text{high}}$ CD25$^{\text{+}}$ versus CD45RB$^{\text{low}}$ CD25$^{\text{−}}$ by sorting cells labeled with FITC anti-CD4 (H129.19), PE anti-CD45RB (16A) (BD Pharmingen), and allophycocyanin (APC) anti-CD25 (7D4; eBioscience). Alternatively, B6 mice were first injected i.p. with 250 $\mu$g per mouse of anti-CD25 (hybridoma PC61) MAb 4 days prior to their use as donors for isolation of the CD45RB$^{\text{low}}$ CD25$^{\text{−}}$ CD4 T-cell subset; the efficiency of depletion of the CD45RB$^{\text{low}}$ CD25$^{\text{−}}$ CD4 cells was >90% as monitored by staining with anti-CD25 (7D4). Anti-CD25 (IL-2Rα) MAb used in vivo was generated from hybridoma PC61 (29, 42) and purified via elution over a protein G agarose column (Sigma). The antibody immunoglobulin G (IgG) concentration was determined by comparison to commercial rat anti-mouse CD25 MAb (PC61; eBioscience) in flow cytometric analyses employing FITC-labeled goat anti-rat secondary antibody and indirect immunofluorescence as a standard. Purified rat-IgG1 was used as a normal rat IgG control. Then, spleen cells from these anti-CD25 MAb-treated donor mice were processed in a fashion identical to that described above to yield CD45RB$^{\text{high}}$, CD45RB$^{\text{low}}$, and CD45RB$^{\text{low}}$ CD25$^{\text{−}}$ CD4 T cells.

FIG. 1. Generation of CD4 Th1-like and Th2-like cells. CD62L$^{\text{high}}$ CD44$^{\text{low}}$ T cells were enriched by cell sorting (see Materials and Methods) and cultured in 96-well plates of precoated anti-CD3 and anti-CD28 with a 72-h stimulation. Stimulation was in the presence of IL-12 and anti-IL-4 for Th1 cells and in that of IL-4 and anti-IFN-γ for Th2 cells. Assays utilized for determining the functional differentiation of Th1 and Th2 cells are indicated at the bottom.

![Diagram](https://via.hdl.org/.../2017-7a278gq.png)
with the same pattern of results always obtained.

Either these cells or unfractionated CD4 T cells as a positive control (10^5) were adoptively transferred into B6.nude recipients. Alternatively, we first employed negative selection of CD4 T cells by using a biotinylated MAb cocktail to deplete CD8, CD19, CD11b, and CD11c cells. For the resulting CD4 T-cell population, this procedure was followed by Miltenyi bead positive depletion of CD25^+ CD4 T regulatory (Treg) cells. The purity of the entirely negatively selected flowthrough CD25^+ CD4 T-cell population was ≥99%.

**ELISA determinations of serum Ig.** For measuring hyper-Ig, affinity-purified goat anti-mouse IgM and IgG2a antibody (Southern Biotechnology, Birmingham, AL) was diluted to 7 µg/ml in phosphate-buffered saline (PBS) to coat 96-well ELISA-grade plates (Becton Dickinson, Oxford, CA) overnight at room temperature. The plates were then washed three times with PBS and blocked for 1 h with 5% bovine serum albumin-PBS (Sigma, St. Louis, MO) overnight at room temperature. The plates were then washed three times with PBS and blocked for 1 h with 5% bovine serum albumin-PBS (Sigma, St. Louis, MO) overnight at room temperature. The plates were then washed three times with PBS and blocked for 1 h with 5% bovine serum albumin-PBS (Sigma, St. Louis, MO) at 37°C. Either control uninfected sera or sera obtained at various time points after LP-BM5 infection and at the final termination time point were then plated and allowed to incubate for 2 h at 37°C. The plates were washed three times with PBS, and alkaline phosphatase-labeled goat anti-mouse Ig (Southern Biotechnology) was added. After a 2-h 37°C incubation, p-nitrophenyl phosphate (Sigma) provided a colorimetric change which was then quantitated at 405 nm by an ELISA reader (Dynatech Laboratories, South Hampton, United Kingdom).

**Spleen cell responses to mitogen.** Spleen cells (4 x 10^5/well) from control and experimental mice were plated in triplicate into 96-well plates with medium containing 5% fetal calf serum, l-glutamine, antibiotics, and a final concentration of 10 µg/ml lipopolysaccharide (LPS). After 72 h, wells were pulsed with 1 µCi of [3H]thymidine (Dupont NEN, Boston, Mass.) and harvested 6 h later onto Unifilter 96-well GF/C plates for assessment of thymidine incorporation by scintillation counting (Packard MicroSant NXT counter).

**Flow cytometry.** Spleen cells were incubated with FITC-, PE-, APC-, or Cy-Chrome-conjugated antibodies, and the resulting direct immunofluorescence analysis was used by use of a FACScalibur flow cytometer (BD Bioscience) to detect the expression of murine CD4 (H129.19), CD45RB (16A), CD25 (7D4 or PC61), CD8α (53-6.7), Thy1.2 (53-2.1), B220 (RA3-6B2), CD19 (1D3), CD11b (M1/70), CD11c (HL3), CD62L (MEL-14), CD44 (IM7), IFN-γ (XMG1.2), and IL-4 (11B11) (BD Pharmingen or eBioscience). Appropriate FITC-, PE-, APC-, or Cy-Chrome-conjugated Ig isotypes of irrelevant specificity were used to control for each experimental MAb. ICS of IFN-γ and IL-4 was performed as previously described (27). Briefly, for ICS, cells were cultured with 50 ng/ml phorbol myristate acetate (Sigma) and 1 µg/ml ionomycin (Sigma) for 5 h, with 5 µg/ml brefeldin A added for the last 2 h. Extracellular staining with FITC anti-CD4 was performed and followed by intracellular staining with PE anti-IFN-γ and APC anti-IL-4. Stained cells were analyzed on a FACScalibur flow cytometer using CellQuest software (BD Bioscience).

**RNA isolation and real-time quantitative RT-PCR.** Viral load was determined for BM5de and BM5eco as previously described (9, 16). Briefly, total RNA was isolated from spleen tissue using Tri-Reagent (Molecular Research Center, Cincinnati, OH) and treated with a DNA-free kit (Ambion, Austin, TX). Following reverse transcriptase amplification of cDNA (Bio-Rad iScript cDNA synthesis kit), quantitative reverse transcriptase PCR (RT-PCR) was performed using iQ SYBR green supermix and iCycler software (Bio-Rad, Hercules, CA).

**RESULTS AND DISCUSSION**

Adoptive transfer of CD4 T cells converts MAIDS-resistant B6.nude recipients to a MAIDS-susceptible phenotype. We first compared positive and negative selection methods of purifying mature splenic CD4 T cells from normal donor C57BL/6 mice by experiments utilizing adoptive transfer of CD4 T cells into B6.nude recipients. The mice were infected with LP-BM5 virus i.p. 48 h after reconstitution, and progression to MAIDS was evaluated 9 to 11 weeks later. The following standard readouts of MAIDS-associated symptoms, which we and others have previously established, were employed (16–20, 23, 37, 39): (i) spleen size, with enlargement indicating MAIDS-associated B- and T-cell lymphoproliferation; (ii) serum IgG2a and IgM levels, with increases due to MAIDS polyclonal B-cell activation; and (iii) splenic B-cell responses to LPS stimulation, with decreases indicative of B-cell-associated MAIDS-induced immunodeficiency. For all the experiments, an early activational MAIDS disease parameter, hypergammaglobulinemia (serum IgM and IgG2a levels), was also assessed at about 7 to 8 weeks postinfection (p.i.) by analysis of serum taken without sacrifice of the mouse. We routinely repeated the hyper-Ig determination and added cell-based MAIDS parameters when we terminated the experiments.

In an initial experiment, purified mature splenic CD4 T cells obtained by either positive or negative selection mediated MAIDS by all standard readouts when they were adoptively transferred into B6.nude recipients subsequently infected with LP-BM5. There were no substantial differences in the patterns of results obtained with the positive-selection and negative-selection approaches, and the results were also comparable to those observed for intact B6 mice. Here, for the sake of brevity, we show only one activational parameter of MAIDS (spleen weight) (Fig. 2A) and one immunosuppressive parameter (LPS mitogen response) (Fig. 2B), although all disease readout assays were performed and showed the same pattern of results.
Considering that these were reproducible results in repeat experiments, these data confirmed that positive selection of CD4 T cells was a valid approach for use in additional adoptive transfer experiments to define their required characteristics to mediate LP-BM5-induced disease. Also of note is the fact that viral loads for both the BM5def and BM5eco viral constituents of LP-BM5 were determined at the termination of this kind of experiment. Importantly, there was no reproducible difference between the viral load for infected B6.nude mice that received adoptive transfer of CD4 T cells and that for those mice that did not receive such reconstitution for either the BM5def virus or the BM5eco virus (data not shown). In addition, this viral expression level was similar to that observed for infected wild-type B6 mice (see Fig. 9 and the discussion of viral load below).

The relative contributions of Th1 and Th2 CD4 T-cell functional subsets in MAIDS pathogenesis. A large body of evidence has indicated the existence of functionally polarized CD4 T-cell responses based on their profiles of cytokine secretion, and CD4 T cells have thus been divided into the Th1 and Th2 subpopulations. It has generally been accepted that a Th1/Th2 imbalance underlies various immune diseases. In HIV-infected patients and LP-BM5-infected mice, T-cell proliferation and Th1 cytokine (IL-2 and IFN-γ) production have been reported to decline, while Th2 cytokine (IL-4, IL-5, IL-6, and IL-10) and immunoglobulin production increase (4, 5, 10, 12, 26, 34, 45, 49, 57).

However, whether Th1 and/or Th2 cells and their associated cytokines are critical for the induction of MAIDS pathogenesis remains unknown. In this study, we directly tested the possible requirement for Th1 versus Th2 CD4 T cells in vivo by cell transfer with Th1, Th2, or control CD4 T cells showed significant disease (\( * \), \( P \leq 0.05 \)) by the Student \( t \) test compared with uninfected mice. Furthermore, no significant difference (\( * \), \( P \geq 0.05 \)) was observed in the disease measurements among the three infected groups that received cell transfers, with the Bonferroni adjustment applied as a multiple comparisons posttest. The data shown are representative of three total experiments, with the same pattern of results always obtained.

![FIG. 3. CD4 Th1 and Th2 cells equally mediate MAIDS. Naive CD62L\(^{high}\) CD44\(^{low}\) CD4 T cells were purified from normal B6 splenocytes before Th1 and Th2 skewing in vitro. The subsets were verified by IFN-γ (Th1) and IL-4 (Th2) production, as determined by intracellular cytokine staining (A) and ELISA (B). CD62L\(^{high}\) CD44\(^{low}\) Th1, Th2, or control nonskewed CD4 T cells (1 \( \times \) 10\(^5\)) were adoptively transferred into B6.nude recipients. At 9 weeks p.i., mice were assayed for disease by examination of hypergammaglobulinemia via ELISA measurement of serum Ig levels (C) and of immunodeficiency via lymphocyte proliferation following LPS stimulation for 72 h (D). To depict the uninfected control, the data from all groups of uninfected mice receiving the different transfers of experimental cell populations were pooled for simplicity because the values were very similar. With both disease parameters, mice reconstituted with Th1, Th2, or control CD4 T cells showed significant disease (\( ** \), \( P \leq 0.05 \)) by the Student \( t \) test compared with uninfected mice. Furthermore, no significant difference (\( * \), \( P \geq 0.05 \)) was observed in the disease measurements among the three infected groups that received cell transfers, with the Bonferroni adjustment applied as a multiple comparisons posttest. The data shown are representative of three total experiments, with the same pattern of results always obtained.](http://jvi.asm.org/)

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By this approach, highly polarized IFN-γ-producing Th1 or IL-4-producing Th2 responses were generated as assayed by both intracellular cytoplasmic staining and flow cytometry (Fig. 3A) and IL-4 and IFN-γ protein secretion as determined by ELISA (Fig. 3B). Th1 and Th2 CD4 T cells were adoptively transferred into B6.nude recipients, which were then subjected to LP-BM5 infection. Progression to full MAIDS was evaluated by sacrificing the mice and assessing the standard readouts for MAIDS. However, the MAIDS disease activation parameter hyper-IgM and -IgG2a levels (Fig. 3C) and LPS responsive-splenomegaly (B). These results were highly consistent with the data obtained for other disease parameters (data not shown), and showed that, similar to infected wild-type intact B6 mice, recipients reconstituted with either Thy1.2− or total CD4 T cells developed similar levels of MAIDS (*, P = 0.05). The data shown are representative of two total experiments, with the same pattern of results always obtained.

Are Thy1.2− CD4 cells central to MAIDS pathogenesis? A CD4 T-cell subset that uniquely expands upon LP-BM5-induced MAIDS pathogenesis was next examined to determine if it plays a causal role in this disease. Of the CD4 T cells derived from the B6 mice that are utilized here as the prototypic MAIDS-susceptible strain, all are also Thy1.2+, except for about 1% which are Thy1.2−. A report from the laboratory of Morse and coworkers (24) demonstrated, however, that this small Thy1.2− CD4 T-cell subpopulation increased substantially following LP-BM5 infection. We have previously confirmed this finding (16), e.g., the ratio of Thy1.2− CD4 among total CD4 T cells increased from 5% (Fig. 4A, left) to 42% (Fig. 4A, right) after LP-BM5 infection. To test for a possible functional role for this rare CD4 T-cell subset in MAIDS induction, we purified Thy1.2+ CD4 T cells (Fig. 4A, bottom) and thereby deleted Thy1.2− CD4 T cells from B6 donors (Fig. 4A, left). After adoptive transfer of only the purified Thy1.2− CD4 T cells into B6.nude recipients and subsequent LP-BM5 infection, we assessed MAIDS pathogenesis by spleen weight (Fig. 4B) and all the other standard MAIDS readouts (data not shown). It was clear that there was no significant difference between the B6.nude recipients reconstituted with total CD4 T cells and those reconstituted with Thy1.2− CD4 T cells only. Furthermore, at the termination of the experiment, flow cytometric analysis indicated that there was no LP-BM5 infection-dependent increase of Thy1.2− CD4 T cells from the very small percentages of such contaminating cells in B6.nude mice receiving purified Thy1.2+ CD4 T cells. This lack of increase (i.e., 0.9-fold) of the average percentage of Thy1.2− CD4 T cells (of total CD4 T cells) is to be contrasted with the 3.7-fold increase in the Thy1.2− T-cell level for the infected Bb.nude group reconstituted with total CD4 T cells over that for the noninfected but similarly reconstituted group. Thus, because purified Thy1.2+ CD4 T-cell transfer leads to an equivalent level of disease (Fig. 4B), there was no evidence that Thy1.2− CD4 T cells played a key role in MAIDS pathogenesis. In addition, it is not clear whether the increase in the Thy1.2− CD4 T-cell subpopulation is a result of an actual expansion of these cells or whether it (also) involves Thy1.2 downregulated expression on previously Thy1.2+ cells.

Do the required CD4 T cells need to be of the CD45RBhigh/naive or the CD45RBlow/memory phenotype to mediate LP-BM5 induction of MAIDS? In this series of experiments, the goal was to focus on whether previous antigen experience acquired through the T-cell receptor governs the pathogenic ability of CD4 T cells in MAIDS. To this end, we separated CD45RBhigh/naive CD4 cells and CD45RBlow/memory CD4 cells from normal B6 mice splenocytes and adoptively transferred such purified subsets into B6.nude recipients. MAIDS disease parameters were again assessed both at about 7 to 8

![FIG. 4.](http://jvi.asm.org/)}
weeks p.i. by analysis of serum and at the 9- to 11-week terminal time point by use of spleen cell readouts.

Positively selected total CD4 T cells (Fig. 5A, left) from normal B6 splenocytes were subsequently stained with CD45RB-PE. The CD45RB\textsuperscript{high} and CD45RB\textsuperscript{low} populations were empirically defined as the brightest-staining 30% and dullest-staining 15%, respectively, of the CD4 cells (see Materials and Methods). The purities of the postsorted and pretransferred CD4 T-cell populations used were both $\geq 98\%$. Equal numbers ($5 \times 10^6$) of CD4 T cells were adoptively transferred into B6.nude recipients. Disease parameters were determined at 9 weeks p.i. by assessment of hypergammaglobulinemia via ELISA measurement of serum IgM and IgG2a (B) and of immunodeficiency via lymphocyte proliferation in response to LPS (C). Both CD45RB\textsuperscript{high} and CD45RB\textsuperscript{low} CD4 T cells mediated MAIDS compared with the uninfected control ($**$, $P \leq 0.05$); however, compared to B6.nude mice receiving CD45RB\textsuperscript{high} or unfractionated CD4 T cells, recipients reconstituted with CD45RB\textsuperscript{low} cells mediated significantly less disease by the Student $t$ test ($**$, $P < 0.05$), with the Bonferroni adjustment applied as a multiple comparisons posttest. These results were consistent with all other disease parameters (data not shown). The data shown are representative of two experiments, with the same pattern of results always obtained.

FIG. 5. CD4 CD45RB\textsuperscript{low} cells mediate MAIDS, but to a lesser degree than CD45RB\textsuperscript{high} CD4 T cells. Purified CD4 T cells (A, left) from normal B6 donors were sorted into 30% brightest CD45RB\textsuperscript{high} (A, upper right) and 15% dullest CD45RB\textsuperscript{low} (A, lower right) CD4 T cells. Equal numbers ($5 \times 10^6$) of CD45RB\textsuperscript{high}, CD45RB\textsuperscript{low}, or total unfractinated (as a positive control) CD4 T cells were adoptively transferred into B6.nude recipients. Disease parameters were determined at 9 weeks p.i. by assessment of hypergammaglobulinemia via ELISA measurement of serum IgM and IgG2a (B) and of immunodeficiency via lymphocyte proliferation in response to LPS (C). Both CD45RB\textsuperscript{high} and CD45RB\textsuperscript{low} CD4 T cells mediated MAIDS compared with the uninfected control ($**$, $P \leq 0.05$); however, compared to B6.nude mice receiving CD45RB\textsuperscript{high} or unfractionated CD4 T cells, recipients reconstituted with CD45RB\textsuperscript{low} cells mediated significantly less disease by the Student $t$ test ($**$, $P < 0.05$), with the Bonferroni adjustment applied as a multiple comparisons posttest. These results were consistent with all other disease parameters (data not shown). The data shown are representative of two experiments, with the same pattern of results always obtained.

Compared to uninfected mice that received control CD4 T cells, both CD45RB\textsuperscript{low} and CD45RB\textsuperscript{high} CD4 T cells clearly mediated LP-BM5-induced MAIDS. Interestingly, however, the extent of disease consistently seemed to depend on the CD45RB status of the transferred CD4 T cells. For example, the hyper-IgM and -IgG2a levels of the B6.nude recipients reconstituted with the CD45RB\textsuperscript{low} CD4 cell group were significantly lower than those of the B6.nude recipients reconstituted either with total CD4 or with CD45RB\textsuperscript{high} CD4 T cells (Fig. 5B). Similarly, the CD45RB\textsuperscript{low} CD4 T-cell-mediated re-
sidual mitogenic response to LPS of the B6.nude recipients reconstituted with CD45RB low CD4 cells was significantly higher than that of the B6.nude recipients reconstituted with either total CD4 or CD45RB high CD4 T cells (Fig. 5C). In contrast, donor CD45RB high cells mediated the immunodeficiency of MAIDS after LP-BM5 infection to an extent similar to that seen following transfer of unfractionated CD4 T cells. Thus, CD45RBlow CD4 cells supported MAIDS but not to the same degree as CD45RB high CD4 cells; this effect was observed in the two experiments in which only these subsets were compared and also in the subsequent more complex experiments, in which the CD45RBlow subset was further fractionated.

Do CD45RB low CD25+ CD4 T- regulatory cells inhibit MAIDS development? One interpretation of the results given above is that CD45RB low CD4 cells intrinsically support significantly less MAIDS development than CD45RB high CD4 T cells. An alternative reason that the CD45RB high versus CD45RBlow CD4 T-cell subsets might differentially support LP-BM5-induced MAIDS is that the latter memory CD4 T-cell population uniquely contains the natural Treg (nTreg) subset. Perhaps these nTreg cells limit the extent of LP-BM5-induced disease, either (i) as anergic cells by “diluting” the CD45RBlow CD4 T-cell population and thus decreasing the percentage of non-nTreg CD25+ CD4 cells that are able to mediate MAIDS pathogenesis present or (ii) by actively inhibiting or suppressing the ability of the CD25+ CD4 T cells to undergo activation and proliferation upon LP-BM5 infection and thus to promote viral pathogenesis.

Indeed, our experiments showed that in normal naive, uninfected B6 mice, about 8.9% of the total CD4 population consisted of CD25+ nTreg cells (Fig. 6A, left). In contrast to the starting distribution of Tregs, among the postsorted CD45RB high CD4 T cells (Fig. 6A, middle), only 2.6% of cells were CD25+, whereas there were 16.7% CD25+ Treg cells among postsorted CD45RB low CD4 T cells (Fig. 6A, right).

To address the possible negative role of nTreg cells in LP-BM5-induced viral pathogenesis, we depleted CD25+ CD4 T cells from the CD45RB low population. In addition to the unfractionated CD4 T cells and the CD45RB high and CD45RBlow subsets purified as described above, a CD45RBlow population depleted of CD25+ CD4 T cells was adoptively transferred in parallel into B6.nude recipients. In the representative experiment shown in Fig. 6, we first confirmed, as for the experiment shown in Fig. 5, the result that the extent of disease consistently depended on the CD45RB status of transferred CD4 T cells. In addition, there was no evidence that the CD45RBlow CD25+ nTreg population was able to moderate the ability of CD45RBlow CD4 T cells to mediate MAIDS. The spleen weight (Fig. 6B), hyper-IgM level (Fig. 6C), and mitogenic
response to LPS (Fig. 6D) results showed that the CD45RB<sub>low</sub>, CD45RB<sub>low</sub> CD25<sup>+</sup>, and CD45RB<sub>low</sub> CD25<sup>-</sup> reconstituted groups were similar to each other. Further, these groups all mediated significantly less MAIDS development than either the unfractionated CD4 group or the CD45RB<sub>high</sub> group.

To further limit any contamination of nTreg cells in the CD25<sup>-</sup>-depleted CD45RB<sub>low</sub> preparation, we treated donor B6 mice with anti-CD25 MAb in vivo 4 days prior to purification of CD45RB<sub>high</sub>, versus CD45RB<sub>low</sub>, CD4 T cells and subsequent further separation. After again obtaining a highly enriched (>98% pure) CD25<sup>-</sup> CD4 cell population, we repeated the adoptive transfer of un fractionated CD4, CD45RB<sub>high</sub>, CD45RB<sub>low</sub>, or CD45RB<sub>low</sub> CD25<sup>+</sup> cells into B6.nude recipients. One of the representative activation assay parameters spleen weight (Fig. 7A), and one immunodeficiency parameter, mitogenic response to LPS (Fig. 7B), are depicted, although these and all other disease readouts showed a pattern of results identical to that shown in Fig. 6 when the B6 donor mice were employed without in vivo anti-CD25 treatment prior to the CD45RB purification. Thus, depletion of CD25<sup>+</sup> nTreg cells from the CD45RB<sub>low</sub> CD4 subset did not improve the ability of the cells in this subset to mediate LP-BM5-induced MAIDS.

Possible requirement for nTreg cells in MAIDS. Because there was no evidence for the involvement of CD45RB<sub>low</sub> CD25<sup>+</sup> nTregs in modulating the extent of MAIDS pathogenesis, while some CD4 CD25<sup>+</sup> potential nTregs are CD45RB<sub>high</sub> (Fig. 6A) (13), it was important to determine whether this nTreg subpopulation regulates viral pathogenesis in the context of the naive, unmanipulated CD4 T-cell population. Thus, CD4 T cells isolated from normal B6 splenocytes (Fig. 8A, left) were stained with FITC anti-CD25 and PE anti-CD4 and sorted such that CD25<sup>-</sup> CD4<sup>-</sup> T cells were collected, free of CD25<sup>+</sup> nTreg CD4<sup>-</sup> T cells. The purity of CD25<sup>-</sup> CD4<sup>-</sup> T cells was >99% (Fig. 8A, right). These CD25<sup>-</sup>-depleted CD4 T cells, with un fractionated total CD4 T cells used as a positive control, were adoptively transferred into B6.nude recipients, and disease susceptibility was assessed as usual at 9 to 11 weeks p.i.

Measurements by all the MAIDS readouts provided the same result: adoptive transfer of either total CD4 T cells or CD25<sup>-</sup> CD4<sup>-</sup> T cells into B6.nude mice led to essentially identical amounts of disease. As examples of the MAIDS readouts, one activation parameter, spleen weight (Fig. 8B), and one immunosuppressive parameter, LPS response (Fig. 8C), are depicted as representative findings.

To confirm these results and to further decrease the possibility that a minor fraction of persisting CD25<sup>+</sup> nTregs was present and sufficient to limit the extent of disease, we employed an alternative approach to perhaps more completely eliminate the CD25<sup>-</sup> CD4 Treg cells. Thus, rather than performing flow cytometric sorting, we depleted the CD4 CD25<sup>-</sup> nTreg cells by use of Miltenyi anti-CD25 beads. Again, however, there was no difference in the abilities of the CD25<sup>-</sup>-depleted CD4 T cells and the un fractionated CD4 T cells to mediate MAIDS (data not shown). Thus, using several approaches collectively, we found no evidence that CD25<sup>-</sup> CD4 nTreg cells played an important role in MAIDS pathogenesis, including that in the context of the total CD4 T-cell population.

A decrease in disease pathogenesis mediated by CD45RB<sub>low</sub>

CD4 T cells does not correlate with a reduction in viral load.

To determine if the observed decrease in the extent of LP-BM5-induced MAIDS pathogenesis mediated by CD45RB<sub>low</sub> CD4 T cells might be due to a reduced viral load, the expression levels of the pathogenic BM5def and helper BM5eco viruses were determined by quantitative RT-PCR assay (9, 16) for the experiments shown in Fig. 5 through 7 with the representative data from Fig. 6 as shown here. By this assay, the levels of the BM5def and BM5eco viruses were not detectable in uninfected mice, as expected (Fig. 9). Infected B6.nude recipients that received previous transfer of total CD4 T cells expressed high defective and ecotropic viral load levels, which were approximately the same as those of the infected B6 group. B6.nude mice receiving adoptive transfer of either CD45RB<sub>low</sub>, CD45RB<sub>low</sub> CD25<sup>-</sup>, or CD45RB<sub>low</sub> CD25<sup>+</sup> CD4 T cells, all of which mediated an extent of MAIDS pathogenesis lesser than that of the CD45RB<sub>low</sub> CD4 subset (Fig. 6 and 7), expressed approximately identical BM5def and BM5eco viral load levels at 10 weeks after LP-BM5 infection. Impor-
tantly and surprisingly, B6.nude recipients that received CD45RB<sup>high</sup> CD4 T cells, which mediated substantially more MAIDS pathogenesis (Fig. 6), expressed, if anything, somewhat lower BM5<sup>def</sup> and BM5<sup>eco</sup> viral levels in this experiment, although this trend was not statistically significant (Fig. 9). In the other experiments (Fig. 5 and 7), the levels of BM5<sup>def</sup> and BM5<sup>eco</sup> mRNA expression were even more similar between the mouse groups receiving CD45RBlow cells and/or CD45RB<sub>low</sub> subsets and those receiving CD45RB<sub>high</sub> CD4 T cells. Thus, clearly the reduced ability of CD45RB<sub>low</sub> CD4 cells to mediate LP-BM5-induced MAIDS could not be ascribed to a reduced ability of LP-BM5 to infect, or of the component viruses to be expressed in, B6.nude mice that received these calls by adoptive transfer.

**Implications of the present study.** In the present study, we sought to define the critical cell surface phenotypic and/or functional characteristics of CD4 T cells, whose expression allowed these T cells to mediate MAIDS pathogenesis upon infection of susceptible B6 mice with the LP-BM5 retroviral isolate. Thus, beyond the crucial ability to upregulate CD154 (CD40L) expression by these pathogenic CD4 T cells such that CD40<sup>+</sup> B cells are activated and transmit the CD40-TRAF-6-dependent signals necessary for disease induction (16–20), the function of CD4 T cells in mediating MAIDS is incompletely understood.
understood. Included in the unresolved issues is the question of whether the functional/phenotypic parameters that have been observed for CD4 T cells obtained from mice with ongoing MAIDS—such as differential cytokine production, anergic characteristics, and altered cell surface properties—are relevant to their required functional role in the induction of MAIDS pathogenesis. Alternatively, these changes simply may be markers of the effects of established disease on the CD4 T-cell compartment.

To separate the requirements for MAIDS induction that operate at the level of the necessary CD4 T-cell population from possible MAIDS epiphenomena, we utilized an adoptive transfer approach to directly compare the abilities of different CD4 T-cell subsets to reconstitute B6.nude mice for susceptibility to LP-BM5-induced MAIDS. By use of this approach, some of the characteristics of CD4 T cells from LP-BM5-infected mice with disease, e.g., the lack of expression of the Thy1 (CD90) marker by the MAIDS-expanded unique population of CD4 Thy1− (CD3+) T cells (Fig. 4), were found to likely be irrelevant to CD4 T-cell pathogenic function. Thus, there was no effect of the removal of Thy1− CD4 T cells; rather, this depleted CD4 T-cell population mediated full-blown MAIDS upon LP-BM5 infection.

Similarly, we found no consistent difference in the abilities of CD4 T-cell populations that were skewed to the Th1 versus Th2 functional subsets to mediate LP-BM5-induced MAIDS in the adoptive transfer model (Fig. 1 and 3). Consideration of a possible differential role of Th1 versus Th2 cells as the required pathogenic CD4 T cells was pertinent, given the existing literature on both cytokine production specifically in MAIDS (11, 35, 36, 38) and the critical function of Th1/Th2 CD4 T cells in various other disease states. Thus, in addition to playing differential roles in protection, polarized Th1-type and Th2-type responses are also responsible for different types of immunopathological reactions. Th1 cells are involved in the pathogenesis of several organ-specific autoimmune disorders. In contrast, allergen-specific Th2 responses may be critical to atopic disorders in genetically susceptible individuals, and skewing to a Th2 response in certain infections diseases, such as leishmaniasis, leprosy, and others, results in a lack of clearance of the causative microbe and consequently in enhanced microbial and/or immunological pathology (4, 34, 45, 57).

More germane to the present murine retroviral immunodeficiency system is the fact that, for HIV type 1 infection, the potential role of cytokines in the development of AIDS has received a great deal of attention. There is some evidence suggesting that a Th2-type cytokine response is often associated with progression to AIDS in HIV-positive individuals (4, 5, 10, 12, 34, 49, 57). Thus, it seemed important to study here the question of whether the Th1 and Th2 subsets were differentially able to mediate LP-BM5-induced MAIDS pathogenesis. However, we could find no evidence to support this possibility. In experiments utilizing adoptive transfer of Th1 or Th2 CD4 T-cell functional subsets, with appropriate differential IFN-γ versus IL-4 cytokine profiles confirmed as skewed in vitro (Fig. 3A and B), into B6.nude recipients, both subsets mediated MAIDS induction equally (Fig. 3C and D). This result, obtained by direct comparison of Th1 T cells to Th2 CD4 T cells, was important given the conflicting results obtained in the past on Th1 versus Th2 involvement in MAIDS. In particular, many previous studies attempted to imply a disease-causing role for Th2 CD4 T cells simply by correlating Th2 cytokine production during ongoing MAIDS. Our result is in keeping with two other studies that also utilized more-direct approaches to examine the need for Th2 T cells in MAIDS: these studies found (i) that when mice with a genetic disruption of the IL-4 gene, but specifically fully backcrossed onto the susceptible B6 background, are used, MAIDS develops normally following LP-BM5 infection (35, 36), and (ii) that B6 mice deficient in STAT6, which is the proximal signal transducer of ligated IL-4 receptor, also exhibited normal susceptibility to the induction of MAIDS (38).

For some of the other functional/phenotypic parameters of CD4 T cells, the results and their implications were more complicated and deserve a more detailed consideration. These issues include the level of expression of CD45RB by the pathogenic CD4 T cells and the possible involvement of CD25+ CD4 nTregs. Our results from comparison of CD45RBhigh naive/ non-antigen-experienced to CD45RBlow memory/antigen-experienced CD4 T cells indicated that while both functional subsets supported MAIDS induction and progression, only the CD45RBhigh subgroup mediated full disease after LP-BM5 infection (Fig. 5B and C). In contrast, the CD45RBlow memory subset consistently (Fig. 5, 6, and 7) supported only 40% to 55% as much pathogenesis, and this was consistent across all MAIDS disease parameters. This decreased severity of MAIDS was not due to a reduced ability of the B6.nude mice receiving a CD45RBlow CD4 T-cell transfer to become infected by, or to express, the LP-BM5 retrovirus. Thus, quantitative real-time PCR analysis clearly indicated that both the pathogenic BM5def and the helper BM5eco component viruses were expressed at least as well in the CD45RBlow recipient mice (Fig. 9). Therefore, either or both of the following two explanations seemed most likely. (i) CD45RBlow CD4 T cells have intrinsic properties on a per-cell basis that prohibit them from being able to fully support MAIDS induction, and/or (ii) the CD25+ CD4 natural Treg population largely contained within the CD45RBlow compartment inhibits the ability of the pathogenic effector CD4 T cells also in this compartment to mediate LP-BM5-induced MAIDS.

The possibility that nTreg cells were involved was tested first, particularly as their relatively recent identification has led to an explosion of literature reports demonstrating their critical involvement in the regulation of T-cell responses, including in a variety of disease states (43, 50, 51, 53). In short, a subpopulation of normal CD4 T cells that coexpresses the inter-leukin-2 receptor (IL-2R) α chain (CD25) has been defined as a suppressive nTreg population that appears to play an important function in controlling the development of autoimmune diseases and other immune responses, including Th1/inflammatory-type phenomena (43, 51, 53). The naturally occurring murine CD45RBlow CD25+ CD4 nTreg cells have been shown to prevent T-cell-mediated immune pathology in several disease models, especially autoimmune processes, and there is now evidence that CD25+ CD4 nTregs may be involved in many other aspects of immunoregulation, including the responses to autoantigens, alloantigens, tumor antigens, and pathogen-derived antigens and even the maintenance of normal pregnancy (14, 31, 32, 40, 44, 48, 50, 51, 54).

Although other cell surface markers have been associated
with various Treg cell populations, including GITR (glucocorticoid-induced tumor necrosis factor receptor) and CTLA-4 (30, 53, 56), none is truly specific for Treg cells, as these markers can also be expressed by activated CD4+ CD25+ T-effector cells (51). CD25 has been and continues to be the most prominent and defining marker for specifically nTreg cells. As a cell surface marker, CD25 is very useful for obtaining cell preparations that are highly enriched for, or depleted of, Treg cells in order to analyze their function. Thus, although FoxP3 has recently emerged as the best marker of functional Treg cells, it is a transcription factor whose identification by cytoplasmic staining does not lend itself to purification of viable cells for functional testing (53).

In the present study, we addressed the possible regulatory role of nTreg cells in MAIDS by use of an adoptive transfer system featuring B6.nude recipient mice. Naive mouse donor sources of pathogenic CD4 T cells, either (i) the CD45RBlow subset that mediates partial LP-BM5 retrovirus-induced MAIDS pathogenesis relative to the CD45RBhigh subset or (ii) the unfractonated total CD4 population, were efficiently depleted of CD25+ CD4 nTreg cells by several approaches (Fig. 6 through 8). This effectiveness of nTreg depletion coupled with the use of B6.nude recipients provided an appropriate system by which nTreg cells were removed during the entire course of the LP-BM5 infection. Thus, it has been recently confirmed that nTreg development requires selection in an intact thymus (30, 53, 56) and reported that CD11c+ major histocompatibility complex class II+ thymic medullary dendritic cells that are activated by thymic stromal lymphopoietin to express high levels of CD80 and CD86 are required for the induction of the proliferation and differentiation of thymocytes into CD25+ FoxP3+ CD4 nTreg cells (58).

However, despite the use of the B6.nude adoptive transfer system to eliminate nTreg cells, MAIDS developed upon LP-BM5 infection. CD45RBlow CD4 T cells continued to mediate MAIDS pathogenesis at reduced levels whether or not CD25+ CD4 nTregs were present (Fig. 6 and 7), indicating that their decreased pathogenic function was not due to CD45RBlow nTreg suppression. Full-blown MAIDS was also observed despite the depletion of nTregs from the unfractonated naive CD4 population (Fig. 8). Therefore, there was no evidence for the involvement of nTregs in the intact CD4 system. In addition, based on the nearly complete inhibition obtained by blocking anti-CD80/CD86 MAb treatment, it has been reported that nTreg differentiation in the thymus requires CD80/CD86 expression (58). Therefore, our results here favoring a lack of nTreg involvement in MAIDS are supported by our earlier study showing that B6.CD80/CD86 double-knockout mice are MAIDS susceptible (17).

We thus have evidence arguing against the involvement of CD25+ nTregs and any other Tregs that derive from a CD25+ precursor cell. However, we cannot exclude the possible role of other CD4 Treg populations in LP-BM5-induced MAIDS initiation or progression. In addition to CD25+ nTregs and their progeny, there are at least two other distinct CD4 Treg subsets: (i) type 1 T-regulatory cells (Tr1), or "inducible" Tregs (33, 46, 47), and (ii) Th3-type Tregs (1, 30, 43). It is possible that either or both of these Treg cell types may derive from CD25+ CD4 T cells. In particular, given that MAIDS induction stems from retroviral infection, the inducible Tr1 regulatory cells are an attractive candidate. Along these lines, a study by Beilharz et al. (6) reported that MAIDS pathogenesis is partially reduced by in vivo antibody treatment. The antibody injections were given every fourth day beginning at 2 days preinfection and continued to 18 days postinfection. Certain combinations of two or three antibodies injected simultaneously led to a decrease in LP-BM5-induced splenomegaly. In contrast, other combinations, i.e., of a group consisting of MAbs against CD25, CTLA-4, and GITR, or these MAbs used singly, had no significant effect. However, this approach could not distinguish between a blocking effect of these antibodies on Treg cells, as was suggested, versus effects on pathogenic CD4 T effector cells. This is of concern, especially since GITR is known to be a costimulatory receptor for CD4 effector cells in other systems (30, 53). Thus, the possibility of Tr1 Treg involvement in MAIDS demands further consideration. Such future studies will require new reagents and/or approaches that will allow for the separate manipulation of the Treg and pathogenic effector CD4 T-cell compartments.

In summary, our results argue against a role for nTreg cells in MAIDS pathogenesis. Rather, an intrinsic defect in the CD45RBlow CD4 T-cell population is implied as the underlying reason for its reduced MAIDS pathogenic potential in conjunction with LP-BM5 infection. Determining whether it is the antigen-experienced/memory phenotype that is characteristic of this subset per se or other associated phenotypic or functional parameters that form the basis for this difference will require additional experimentation. However, the present results provide further insight into the involvement of CD4 T-cell subsets in this retrovirus-induced disease model and highlight the heterogeneity of this cellular compartment, which is required for MAIDS pathogenesis.

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