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Tissue-Specific Expression of Human CD4 in Transgenic Mice

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The gene for the human CD4 glycoprotein, which serves as the receptor for human immunodeficiency virus type 1, along with approximately 23 kb of sequence upstream of the translational start site, was cloned. The ability of 5′ flanking sequences to direct tissue-specific expression was tested in cell culture and in transgenic mice. A 5′ flanking region of 6 kb was able to direct transcription of the CD4 gene in NIH 3T3 cells but did not result in detectable expression in the murine T-cell line EL4 or in four lines of transgenic mice. A larger 5′ flanking region of approximately 23 kb directed high-level CD4 transcription in the murine T-cell line EL4 and in three independent lines of transgenic mice. Human CD4 expression in all tissues analyzed was tightly correlated with murine CD4 expression; the highest levels of human CD4 RNA expression were found in the thymus and spleen, with relatively low levels detected in other tissues. Expression of human CD4 protein in peripheral blood mononuclear cells was examined by flow cytometry in these transgenic animals and found to be restricted to the murine CD4$^+$ subset of lymphocytes. Human CD4 protein, detected with an anti-human CD4 monoclonal antibody, was present on the surface of 45 to 50% of the peripheral blood mononuclear cells from all transgenic lines.

AIDS is a fatal disease in humans caused by human immunodeficiency virus type 1 (HIV-1) (2, 6), for which there is no known cure at present. With the expectation that HIV will infect 15 to 20 million individuals by the year 2000 (7), the development of vaccines or therapeutics for this disease is of utmost importance. One of the most serious problems in the study of HIV-1 is the lack of a suitable animal model, since the currently used species—chimpanzees, gibbons, and pigtail macaques (1, 5, 16)—are large and expensive to maintain, reproduce slowly, and do not develop any disease symptoms. It is our intention to create a suitable small animal model for HIV-1 infection by using transgene technology.

The receptor for HIV-1 is human CD4, a transmembrane glycoprotein which normally participates in the development and physiology of T cells. Our goal is to express the human CD4 protein on the surface of cells in transgenic animals that normally express CD4, primarily lymphocytes, to render them susceptible to infection by HIV-1. Previous attempts to infect murine cell lines expressing human CD4 from heterologous promoters with HIV-1 have not been successful (17), presumably because of a block in internalization of the virus. However, rabbit cell lines can be infected by HIV-1 (11), and expression of human CD4 on the surface of these cell lines renders them much more susceptible to viral infection (8, 24). Therefore, a transgenic rabbit expressing human CD4 in a tissue-specific manner should prove to be a suitable model for HIV-1 infection.

To investigate the regulation of human CD4 gene expression in a transgenic animal, we have cloned the gene and upstream sequences for human CD4 from genomic libraries and assembled them into expression vectors. These DNA constructs were first analyzed in tissue culture cells for suitable expression patterns. Subsequently, the CD4 vectors were used to produce transgenic mice that express human CD4 in a tissue-specific manner paralleling expression of the endogenous murine CD4. These results suggest that the regulatory sequences required for expression of the human CD4 gene in the proper cells are recognized by murine regulatory factors.

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MATERIALS AND METHODS

Isolation of human CD4 genomic clones. Genomic libraries constructed from placental DNA in the cosmid vector pWE15 (Stratagene) were plated onto Hybond C-extra filters (Amersham) at a density of approximately 25,000 colonies per 137-mm-diameter filter. Replica filters were hybridized overnight at 42°C with a CD4 cDNA (17) probe in 50% formamide–5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7])–5× Denhardt's solution–0.1% sodium dodecyl sulfate (SDS)–100 μg of denatured carrier DNA per ml. After hybridization, the filters were washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature for 10 min and then twice for 30 min at 65°C in 0.2× SSC with 0.1% SDS. Purified clones were obtained by successive rounds of hybridization and screening.

DNAs were prepared from each of the purified clones and digested with *Bam*HI for Southern blot analysis with the CD4 probe described above. The human CD4 gene is contained within five *Bam*HI bands of 20, 6.6, 4.0, 1.8, and 1.0 kb (18), all of which should hybridize to the CD4 cDNA probe. Several overlapping clones containing all or part of the CD4 gene were identified (see Fig. 1). One of these clones, pCD417A.2, contained the complete gene and was selected for further analysis.

Tissue culture and transfections. The murine cell lines NIH 3T3 and EL4 and the human cell line SupT1 (a T-cell line

which expresses high levels of human CD4) were obtained from the American Type Culture Collection and cultured according to specifications. Transfections with NIH 3T3 cells were performed by established calcium phosphate coprecipitation techniques (22). Transfections with the murine T-cell line EL4 were performed by electroporation with the Bio-Rad GenePulser apparatus. For both types of transfections, cell clones were selected in 500 μg of G418 per ml.

Construction of LPCD4. The transgene LPCD4 was constructed from components of cosmid CD42B.1 (see Fig. 1) and from the human CD4 cDNA. The following fragments were excised and assembled in the following order: (i) the 20-kb *Bam*HI fragment of CD42B.1 located upstream of the coding region (see Fig. 1); (ii) a 1.5-kb *Bam*HI-*Rsa*I fragment created by polymerase chain reaction of genomic DNA containing 5′ flanking sequences, exon 1, intron 1, and part of exon 2; (iii) a 1.3-kb *Rsa*I-*Nar*I fragment containing cDNA sequences from exon 2 to exon 7; and (iv) a 1.4-kb *Nar*I-*Eco*RI fragment containing the remainder of the coding sequences and the complete 3′ untranslated region. These fragments were placed in front of the polyadenylation region of simian virus 40 in the cosmid vector pWE15.

Southern and Northern (RNA) blot analysis. DNAs isolated from cell pellets were analyzed by conventional restriction endonuclease cleavage and Southern blotting methods. RNAs were prepared from cell pellets by guanidinium thiocyanate extraction (3), separated by electrophoresis on formaldehyde gels, transferred to Nytran membranes, and hybridized with human or murine CD4 probes. DNA and RNA blots were autoradiographed as described above.

Immunoprecipitations. Immunoprecipitations of metabolically labelled cell lysates were carried out with the antibody OKT4 (Ortho Diagnostics). Cells were pelleted, resuspended at 5×10^6 cells per ml, and labelled for 6 h in the presence of a mixture of [^{35}S]methionine plus [^{35}S]cysteine (New England Nuclear) at a concentration of 200 μCi/ml. Following labelling, the cells were washed twice with phosphate-buffered saline (PBS) and were lysed in Nonidet P-40 lysis buffer (1% Nonidet P-40, 20 mM Tris-HCl [pH 7.2], 150 mM NaCl, 2 μg of aprotinin per ml, and 40 μg of phenylmethylsulfonyl fluoride per ml) on ice for 30 min. The lysates were precleared with mouse immunoglobulin G (IgG) plus protein A-Sepharose for 1 h at 4°C with continuous agitation. The cleared lysates were immunoprecipitated with 5 μl (0.25 μg) of OKT4 overnight at 4°C with continuous agitation. The immune complexes were collected on protein A-Sepharose, washed three times with lysis buffer, and resuspended in 100 μl of Laemmli sample buffer. Prior to electrophoresis, the samples were heated to 95°C for 10 min and then loaded onto a 10% acrylamide gel by the gel system described by Laemmli (12). Following electrophoresis, the gels were fixed in 30% methanol–10% acetic acid for 30 min at room temperature, soaked in En^{3}Hance according to the directions of the manufacturer (New England Nuclear), dried for 90 min, and fluorographed overnight. Soluble human CD4 protein was obtained from American Biotechnologies, Inc.

Antibody staining of cells. Antibody staining was performed on cell lines and on peripheral blood mononuclear cells (PBMCs) obtained from transgenic animals. PBMCs were prepared from 0.5 to 1.0 ml of mouse blood drawn into EDTA or sodium citrate tubes. The blood was first centrifuged for 10 min at 300 × *g* to sediment most of the erythrocytes. The buffy coat was harvested from the interface, diluted to 1.0 ml with PBS, layered over 0.7 ml of Histopaque 1083 (Sigma), and centrifuged for 30 min. Cells harvested from the interface were relatively free of erythrocytes. PBMCs were resuspended at a concentration of 10^7 cells per ml in cold PBS with 2% serum on ice. A total of 10^6 cells were reacted with either 10 μl (0.5 μg) of the anti-human CD4 monoclonal antibody OKT4A-FITC (Ortho Diagnostics) or 4 μl (0.2 μg) of the anti-murine CD4 monoclonal antibody Anti-L3T4-PE (Becton Dickinson) at 4 to 8°C for 30 min. The cells were then washed twice with 2 ml of PBS-serum and resuspended in a minimal volume of the same buffer, and the suspension was observed under fluorescence microscopy. Photographs were taken with Kodak T-Max 400 black and white film. Antibody staining of tissue culture cells was performed as described for PBMCs.

FIG. 1. Genomic cosmid clones obtained by screening human libraries with a human CD4 cDNA probe. B, *Bam*HI site. The *Bam*HI DNA segments are not drawn to scale; the numbers below the line indicate approximate sizes of *Bam*HI fragments in kilobases. Filled boxes represent amino acid coding exons; open boxes represent 5′ and 3′ untranslated mRNA sequences. The 5′ end of the gene is to the left.

Flow cytometry analysis was performed with a FACScan (Becton Dickinson). PBMCs were stained as described above and then fixed in 0.5% paraformaldehyde prepared in Dulbecco's modified Eagle's medium plus 5% horse serum.

Generation and analysis of transgenic mice. The generation of transgenic mice has been discussed in detail elsewhere (9). The mice were purchased from Taconic Laboratories. The DNA constructs used for microinjection were digested with restriction enzymes to remove the cosmid backbone, separated on agarose gels, and purified by electroelution and filtration through Elutip-d columns (Schleicher & Schuell). Murine embryos were obtained from Swiss Webster females mated with B6D2F$_1$ males. Tail biopsy DNA samples from 2- to 3-week-old pups were used for restriction enzyme digestion and Southern blot analysis.

For analysis of CD4 expression, 0.5 to 1.0 ml of blood was drawn from anesthetized mice, and PBMCs were prepared by centrifugation over Histopaque 1083 (Sigma). These PBMCs were used for antibody staining or for RNA preparation and Northern blot analysis. RNAs from various tissues were also prepared by the guanidinium thiocyanate method.

RESULTS

Cloning of human CD4. Cosmid clones containing human CD4 gene sequences were isolated from genomic libraries and are diagrammed in Fig. 1. All initial experiments were performed with cosmid pCD417A.2. This clone contains approximately 6 kb of 5′ flanking sequences, the entire genomic coding and 3′ untranslated regions, and 13 kb of 3′ flanking sequences.

Expression of CD417A.2 in transfected cells and transgenic mice. Cosmid CD417A.2 was linearized with a *Not*I partial digest (*Not*I sites flank the insert in cosmid pWE15) and transfected into NIH 3T3 cells. After 3 to 4 weeks, individual

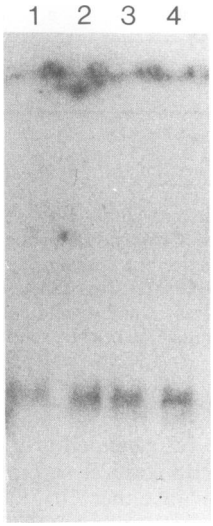

FIG. 2. Analysis of transfected NIH 3T3 cells. RNAs were prepared from four lines of NIH 3T3 cells transfected with CD417A.2 and were blotted and probed with human CD4 cDNA. All four cell lines express a transcript hybridizing with human CD4.

G418-resistant colonies were selected and expanded for further study. DNA and RNA samples were prepared for Southern and Northern blot analyses. All of the cell lines contained the transgene but with various copy numbers (data not shown). The majority (>75%) of the cell lines produced CD4 mRNA, as determined by hybridization to a CD4 cDNA probe (a representative analysis of four cell lines is shown in Fig. 2).

Four lines of transgenic mice carrying the CD417A.2 transgene were generated and were assayed for CD4 expression by antibody staining of isolated PBMCs and by Northern blot and PCR analysis of PBMC RNA. However, no expression of the human CD4 transgene was detected in PBMCs from F_1 animals from any of the lines.

Expression of LPCD4 in transfected cells. The results described above suggested that although the CD417A.2 transgene contained promoter elements with activity in NIH 3T3 cells, the transgene lacked critical elements required for high-level expression of CD4 in the appropriate cell type in mice. Therefore, a second human CD4 transgene containing approximately 17 kb of additional upstream sequence (LPCD4) was constructed. Expression of LPCD4 and CD417A.2 was tested in the murine T-cell line EL4. Each construct was introduced into EL4 cells by electroporation. Cell pools were selected in 500 μg of G418 per ml and were used to prepare DNA and RNA. All of the cell pools contained the appropriate human CD4 transgene DNA as shown in Fig. 3A, lanes 2 to 4. As expected, the CD417A.2 and LPCD4 transgenes have different patterns of hybridizing bands. No human CD4 mRNA was detected in cells containing CD417A.2 (Fig. 3B, lane 2) or in the EL4 control (lane 1). In contrast, the two cell pools containing LPCD4 (Fig. 3B, lanes 3 and 4) produced levels of CD4 mRNA similar to that in the human T-cell line SupT1 (lane 5). Thus, while CD417A.2 failed to be expressed in a murine T-cell background, LPCD4 expresses CD4 mRNA in quantities approximating that of a human T-cell line.

To assess human CD4 protein expression in transfected cells, immunoprecipitation experiments were performed with the human CD4 specific monoclonal antibody OKT4 on

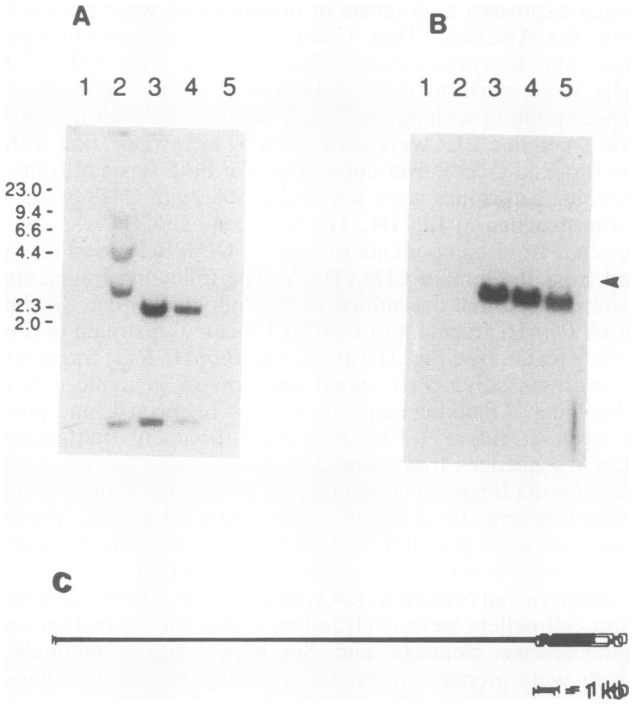

FIG. 3. Analysis of EL4 cells transfected with CD417A.2 or LPCD4. (A) Southern blot of DNAs prepared from EL4 cells (lane 1), EL4 transfected with CD417A.2 (lane 2), EL4 transfected with LPCD4 (lanes 3 and 4), and human genomic DNA digested with *Bam*HI (lane 5). (B) Northern blot of RNA prepared from EL4 cells (lane 1), EL4 cells transfected with CD417A.2 (lane 2), EL4 cells transfected with LPCD4 (lanes 3 and 4), and the human T-cell line SupT1 (lane 5). Samples contained 10 μg of total RNA per lane. The blots in both panels A and B were hybridized to a human CD4 cDNA probe. (C) Diagram of the human CD4 construct LPCD4.

metabolically labelled cell lysates (Fig. 4). As expected from the data described above, lysates from EL4 cells transfected with LPCD4 contain human CD4 protein (Fig. 4, lanes 6 and 7); however, no human CD4 was detected in the lysates derived from EL4 cells transfected with CD417A.2 (lane 5). Preincubation of the antibody with soluble CD4 blocked the reaction, indicating that the immunoprecipitated protein was authentic human CD4 (data not shown). The human cell line SupT1, which expresses abundant amounts of CD4, was used as a positive control in these studies (Fig. 4, lanes 3 and 4 [reacted with mouse IgG or OKT4, respectively]), and untransfected EL4 cells (lanes 1 and 2 [reacted with mouse IgG or OKT4, respectively]) were used as a negative control. Therefore, the murine T cells containing the human CD4 construct LPCD4 express human CD4 at both the mRNA and the protein levels.

To determine whether the human CD4 protein was transported to the cell surface, cells were stained with the anti-CD4 monoclonal antibody OKT4A-FITC. Cells containing LPCD4 expressed cell surface CD4 in amounts comparable to that of the human T-cell line SupT1 (data not shown). Neither the parental cell line EL4 nor cells containing CD417A.2 expressed detectable cell surface CD4.

Expression of LPCD4 in transgenic mice. Three lineages of transgenic mice bearing the LPCD4 transgene were produced. F_1 animals from these lineages have been analyzed for expression of human CD4 in PBMCs. PBMCs were isolated and used to prepare RNA for Northern blot analysis

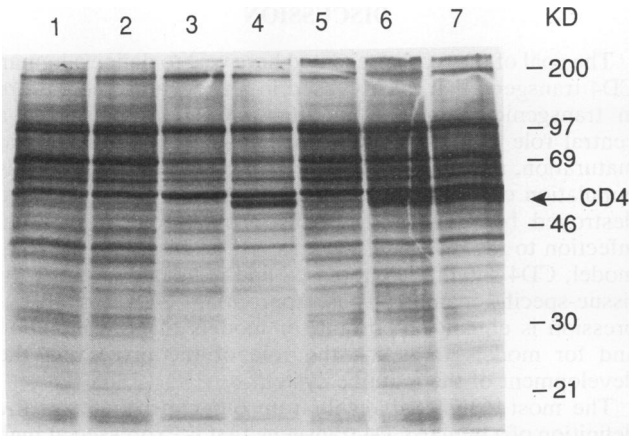

FIG. 4. Immunoprecipitation of ^{35}S-labelled cell lysates. Shown is an autoradiograph of a 10% polyacrylamide gel electrophoresis gel containing EL4 lysates immunoprecipitated with mouse IgG (lane 1) or the anti-human CD4 antibody OKT4 (lane 2), SupT1 lysates immunoprecipitated with mouse IgG (lane 3) or OKT4 (lane 4), lysates of EL4 transfected with CD417A.2 (lane 5), or LPCD4 (lanes 6 and 7) immunoprecipitated with OKT4. Molecular mass markers are shown on the right in kilodaltons.

or were stained with the anti-human CD4 antibody OKT4A-FITC. PBMCs from two animals tested from each of the three lineages produced human CD4 mRNA and cell surface protein. Results from representative experiments are shown in Fig. 5A and B.

Figure 5A shows the result of staining of PBMCs from a lineage no. 0-69 F_1 animal stained with OKT4A-FITC. PBMCs from control animals including nontransgenic littermates exhibited only background levels of OKT4A-FITC staining. PBMCs from all other LPCD4-bearing transgenic animals tested also stained with OKT4A-FITC, although the expression levels among the lineages differed. In each lineage tested, approximately 45 to 50% of PBMCs expressed human CD4 protein. The results of Northern blot hybridization of RNA prepared from PBMCs isolated from four LPCD4 transgenic animals derived from two lineages are shown in Fig. 5B, with human PBMC RNA used as a positive control (lane 6). PBMC RNAs from both lineages shown contain human CD4 mRNA (lanes 2 to 5). Note that the human CD4 mRNA expression levels between individuals from a given lineage are comparable and that the two individuals from lineage no. 0-69 (lanes 2 and 3) express a higher level of human CD4 mRNA than the individuals from lineage no. 0-66 (lanes 4 and 5).

RNA was prepared from various tissues from an F_1 mouse of lineage no. 0-69 to assess the overall pattern of expression of the LPCD4 transgene. Duplicate Northern blots were prepared and hybridized with human (Fig. 6A) or murine (Fig. 6B) CD4 probes to allow a direct comparison of the expression patterns of the endogenous and transgenic CD4 sequences. The tissue distribution of the human CD4 transgene expression parallels that of the murine CD4 gene. The highest levels of expression were observed in the thymus and spleen and in PBMCs; lower levels were observed in the brain, lung, and intestine. Similar results were obtained from analysis of an F_1 animal of lineage no. 0-64. Because these animals were not perfused to remove blood from tissues before sacrifice, it is possible that the low levels of expression seen in some tissues were due to blood cells in the tissue. As previously reported (20), the murine CD4 mRNA in the brain is shorter than in the other tissues.

To determine whether expression of human CD4 was restricted to the murine $CD4^+$ subset of lymphocytes, a quantitative analysis of the PBMC population was carried out by flow cytometry. PBMCs from an F_1 animal were stained with either OKT4A-FITC or anti-L3T4-PE (an anti-murine CD4 antibody) or with both antibodies. The results of

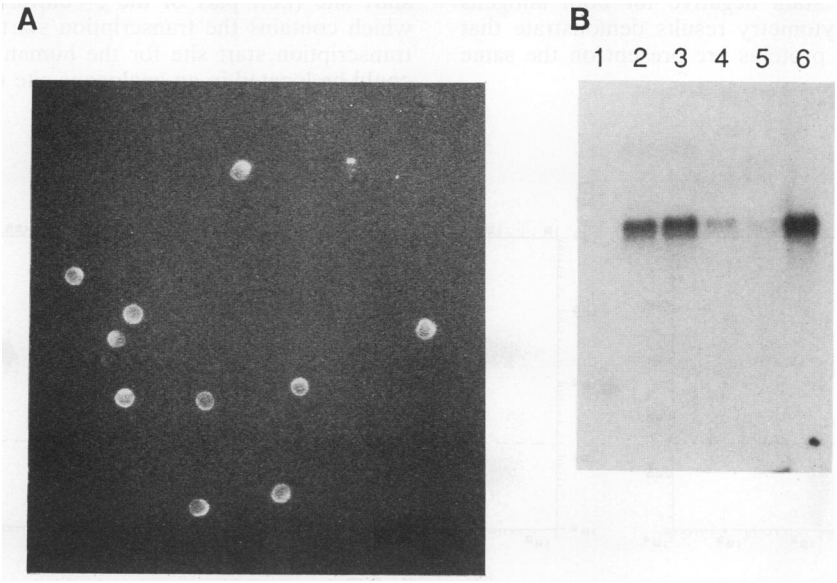

FIG. 5. Expression of human CD4 in PBMCs of transgenic mice. (A) Mononuclear cells of F_1 no. 1-249 were prepared from blood samples and stained with the anti-human CD4 antibody OKT4A-FITC. (B) Northern blot of PBMC RNAs prepared from a nontransgenic mouse (lane 1), lineage no. 0-69 animal (no. 1-242; lane 2), lineage no. 0-69 animal (no. 1-249; lane 3), lineage no. 0-66 animal (no. 1-231; lane 4), lineage no. 0-66 animal (no. 1-259; lane 5), and a human (lane 6) as a positive control. The human sample in lane 6 contains 1 μg of total RNA; the other lanes contain approximately half this amount.

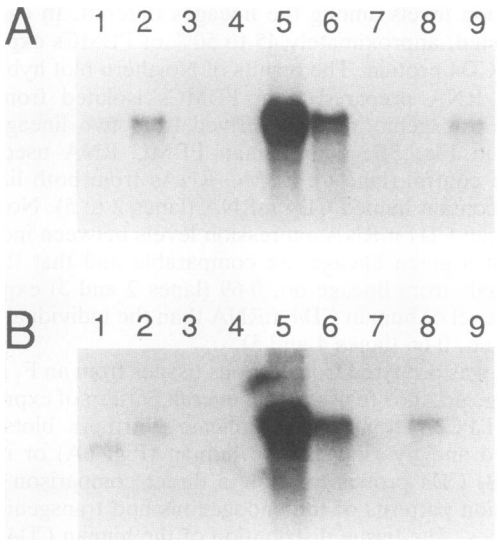

FIG. 6. Tissue-specific expression of human CD4 RNAs in transgenic mice. Tissue RNA samples (5 μg) from animal no. 1-249 (lineage 0-69) were loaded on an agarose-formaldehyde gel, electrophoresed, blotted, and probed with a human CD4 cDNA probe. Samples are from the brain (lane 1), lung (lane 2), liver (lane 3), kidney (lane 4), thymus (lane 5), spleen (lane 6), skin (lane 7), and intestine (lane 8). Lane 9 contains 1 μg of human PBMC RNA as a positive control. Duplicate blots were prepared. The blot in panel A was hybridized with the human CD4 probe, whereas the blot in panel B was hybridized with the murine CD4 probe.

this analysis are shown in Fig. 7. In PBMCs stained with one antibody, approximately 42% of the cells stained positive for human CD4 (Fig. 7A), and 44% of the PBMCs stained positive for murine CD4 (Fig. 7B). The PBMCs stained with both the anti-murine and anti-human antibodies fall into two categories: those which stain positive for both antigens (43%) and those which stain negative for both antigens (56%). Thus, the flow cytometry results demonstrate that murine and human CD4 proteins are present on the same class of lymphocytes.

DISCUSSION

The goal of the work presented here was to define a human CD4 transgene that is expressed in a tissue-specific pattern in transgenic animals. The human CD4 protein plays a central role in HIV infection and in the proper selection, maturation, and physiology of $CD4^+$ helper T cells. It is this population of $CD4^+$ T cells that is selectively targeted and destroyed by HIV-1 during the progression from initial infection to the disease known as AIDS. In an ideal animal model, CD4 should be expressed and regulated in a cell- or tissue-specific manner. Thus, appropriate CD4 protein expression is equally important for models of HIV infection and for models to define the role of the protein in the development of the immune system.

The most significant result from our experiments is the definition of a human CD4 transgene that is expressed at high levels in the appropriate cells in transgenic mice. The expression pattern directed by the CD4 transgene LPCD4 was indistinguishable from the endogenous murine CD4 pattern in the tissues analyzed. The highest levels of expression were seen in PBMCs and in the thymus and spleen, as would be expected from the normal distribution of $CD4^+$ T cells. Most importantly, the human CD4 gene was expressed in the murine $CD4^+$ subset of T cells and was not detectably expressed in the CD4 cell population.

The transgene that was tested, which gives high-level tissue-specific expression of human CD4 in transgenic mice, contains 23 kb of sequence upstream of the amino acid coding exons; a similar construct containing only 5 kb of upstream sequence failed to be expressed in transgenic mice. There are two explanations for the enhancement of transcription in T cells resulting from inclusion of this sequence. One possibility is that an enhancer necessary for tissue-specific expression is present in the additional sequence. A second possibility is that the additional sequence includes a different transcription start site that is used within cells in the animal. In the murine CD4 gene, it has been shown that there is an additional noncoding exon upstream of the translational start site (i.e., part of the 5′ untranslated region [UTR]) which contains the transcription start site (19). The major transcription start site for the human CD4 gene in T cells could be located in an analogous site upstream of the gene,

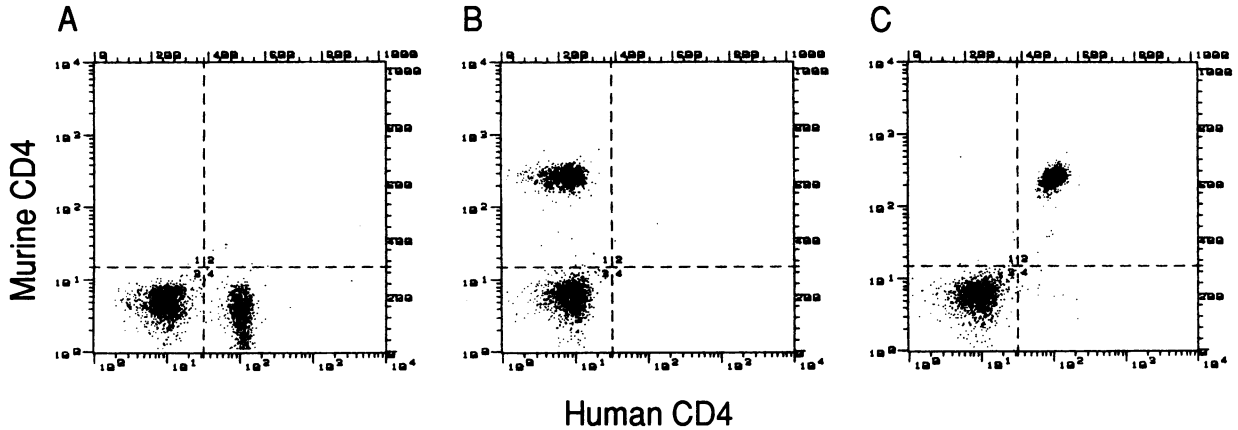

FIG. 7. Flow cytometry analysis of PBMCs from an LPCD4 transgenic F_1 animal of lineage no. 0-69. PBMCs were stained with OKT4A-FITC (A), anti-L3T4A-PE (B), or with both antibodies (C). (A) A total of 42% of the cells stain with OKT4A-FITC (quadrant 4) and 58% are negative for antibody staining (quadrant 3); (B) 44% of the cells stain with anti-L3TA (quadrant 1) and 56% are negative for antibody staining (quadrant 3); (C) 42% of the cells stain with both antibodies (quadrant 2) and 56% stain with neither antibody (quadrant 3).

separated from the amino acid coding sequence by an intron. A direct analysis of the human CD4 mRNA transcription start site(s) would be required to test this hypothesis. However, our data from NIH 3T3 cell transfection experiments demonstrate that a second transcription start site must be located closer to the gene, since 5 kb of upstream sequence direct CD4 gene transcription in this cell line. This would indicate that the CD4 gene has two transcription start sites. Such an arrangement is not without precedent, since the human p^{56}lck gene has been shown to have two promoters: one immediately adjacent to the amino acid coding region and a second one located 34 kb upstream, separated from the rest of the gene by a large intron (23). Preliminary results of deletion derivatives suggest that sequences required for expression in this cell line are located approximately 9 kb upstream from the amino acid coding region (data not shown). Our results from these deletion experiments do not allow us to distinguish between the existence of an enhancer element or of a second transcription start site. More precise analyses of the positions of promoter and enhancer sequences will be required to compare definitively the fine structure of the CD4 genes from mice and humans.

Our original impetus for designing a CD4 transgene that would be appropriately regulated in animals was related to the need for a small animal model for HIV infection. It is likely that the rabbit expressing human CD4 will prove to be the small animal model since rabbits and rabbit cell lines have been shown to be susceptible to HIV-1 infection under certain conditions (4, 10, 11, 21). Furthermore, the addition of human CD4 to the surface of rabbit T-cell lines has rendered them more susceptible to HIV-1 infection (8, 24). Since the human CD4 transgene is appropriately regulated in transgenic mice, it is likely that appropriate CD4 regulation from LPCD4 will be achieved in transgenic rabbits as well. In previous reports, attempts to infect a variety of mouse cell lines expressing human CD4 from heterologous promoters with HIV-1 were not successful, probably because of a block in internalization of the virus (17). It is possible that this is the only point in the viral life cycle that is blocked, since murine cells transfected with HIV-1 DNA (14) and transgenic mice generated from HIV-1 DNA (13) can both produce infectious virus. It should be noted, however, that Locardi et al. (15) have reported successful infection of normal mice with HIV-1 by injecting human cells infected with the virus. The development of a human CD4 transgene which confers tissue-specific expression in transgenic animals is a major step forward in the production of an animal model for HIV infection.

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