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Specific Expression of the Human CD4 Gene in Mature CD4$^+$ CD8$^-$ and Immature CD4$^+$ CD8$^+$ T cells and in Macrophages of Transgenic Mice

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The CD4 protein plays a critical role in the development and function of the immune system. To gain more insight into the mechanism of expression of the human CD4 gene, we cloned 42.2 kbp of genomic sequences comprising the CD4 gene and its surrounding sequences. Studies with transgenic mice revealed that a 12.6-kbp fragment of the human CD4 gene (comprising 2.6 kbp of 5′ sequences upstream of the transcription initiation site, the first two exons and introns, and part of exon 3) contains the sequences required to support the appropriate expression in murine mature CD4$^+$ CD8$^-$ T cells and macrophages but not in immature double-positive CD4$^+$ CD8$^+$ T cells. Expression in CD4$^+$ CD8$^+$ T cells was found to require additional regulatory elements present in a T-cell enhancer fragment recently identified for the murine CD4 gene (S. Sawada and D. R. Littman, Mol. Cell. Biol. 11:5506–5515, 1991). These results suggest that expression of CD4 in mature and immature T-cell subsets may be controlled by distinct and independent regulatory elements. Alternatively, specific regulatory elements may control the expression of CD4 at different levels in mature and immature T-cell subsets. Our data also indicate that mouse macrophages contain the regulatory factors necessary to transcribe the human CD4 gene.

The CD4 gene encodes a transmembrane glycoprotein which is a member of the immunoglobulin gene superfamily (for a review, see reference (19). It is coexpressed with the CD8 molecule on immature thymocytes, early in thymic development, giving rise to double-positive CD4$^+$ CD8$^+$ T cells. The regulation of CD4 and CD8 expression is tightly controlled and tied to the maturation of CD4$^+$ CD8$^+$ T cells. Indeed, in the peripheral immune system, the CD4 and CD8 molecules are expressed on mutually exclusive subsets of mature T cells. CD4 is largely expressed on helper T cells that utilize class II major histocompatibility complex (MHC) proteins as restriction elements for antigen recognition, while CD8 is expressed on cytotoxic and suppressor T cells and interacts with targets expressing class I MHC proteins (19).

The CD4 molecule is essential for normal T-cell functions and plays an important role in T-cell development and activation. It is closely associated with the CD3 T-cell receptor (TcR) complex (29) required for antigen recognition by T cells and for the activation of T cells (2, 29). CD4 is also a signal-transducing molecule which is physically associated with the tyrosine kinase p56^{lck} protein (37). Upon interaction with a specific antigen in the context of MHC class II molecules, CD4$^+$ T lymphocytes produce several lymphokines which induce the proliferation and differentiation of other effector cells of the immune response (19). Therefore, the expression of CD4 on T cells and its interaction with class II MHC proteins is crucial for the development of the cellular immune response.

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In addition, the CD4 protein has been found to be expressed on human monocytes, macrophages, and microglial cells of the central nervous system (10, 39). However, this expression appears to be species specific and is not found in murine monocytes or macrophages (7). A CD4-related transcript has been detected in mouse brain (14, 22), but its cellular site of synthesis has not yet been identified. The function of the CD4 protein in these nonlymphoid cells remains unclear.

Interestingly, the CD4 molecule has been shown to be the main receptor for human immunodeficiency virus (HIV) (8, 17) in both T cells and macrophages. Following HIV infection, CD4$^+$ lymphoid cells lose surface expression of the CD4 molecule, although the precise mechanisms of this down regulation are not known (15).

Thus, establishing the mechanism controlling CD4 expression is important not only because CD4 modulation is a feature of normal T-cell development (19) but also because this molecule is the receptor for HIV. The identification of some of the regulatory elements of the mouse CD4 gene has been reported (34). The CD4 promoter was found, by in vitro transfection studies, to be active through Myb binding sites in mature CD4$^+$ CD8$^-$ T-cell lines but not in immature CD4$^+$ CD8$^+$ T-cell lines (34). Recently, the promoter of the human CD4 gene was characterized, and transcription of the gene was found to be activated by Ets proteins (30). In addition, a T-cell-specific enhancer was identified for the murine (33) and human (3) CD4 genes. However, another group was unable to confirm such an enhancer activity in vitro for the murine sequences (34). Recent studies in transgenic mice have confirmed the presence of this T-cell-specific enhancer (3, 16). It was found that the presence of a T-cell-specific enhancer (3, 16) was necessary to achieve an increased level of T-cell-specific transcription. However, conflicting results

were reported in studies of transgenic mice containing the human CD4 gene and flanking sequences in the absence of the enhancer. One group failed to demonstrate expression of the transgene in T cells, suggesting that the CD4 promoter itself was insufficient for T-cell-specific expression (16), in contrast to the results obtained in vitro (34). Another group was able to show expression in a small fraction of mouse splenocytes, but most of this expression was in B lymphocytes (3). In the same study, the investigators observed expression of human CD4 in the thymus, but this expression was in non-T cells (3). In independent transgenic mice, Gillepsie et al. (13) also identified sequences of the human CD4 gene able to drive expression of the CD4 gene in $CD4^+$ T lymphocytes of peripheral blood mononuclear cells. However, the CD8 marker was not studied, and expression of the transgene in specific T-cell subsets was not reported.

In an effort to understand how the expression of CD4 is controlled during thymocyte development and what factors may be responsible for its complex regulation, we cloned and analyzed the human CD4 gene. Because in vitro transfection studies may not be reliable for identifying the regulatory elements controlling such a complex system, cell-specific expression of the human CD4 promoter/enhancer was studied in transgenic mice. Our results indicate that expression of the human CD4 gene may be controlled by independent regulatory elements active in different subsets of $CD4^+$ T cells. In addition, cellular sequences conferring macrophage-specific expression were identified upstream of the CD4 coding sequences.

MATERIALS AND METHODS

Molecular cloning of genomic CD4 sequences. A human genomic library was prepared essentially as described previously (23, 28). Approximately 10^6 plaques were screened, using the human CD4 cDNA clone (21) as a probe. Eleven positive clones were isolated and mapped by standard single and double digestion with various restriction endonucleases (P. L. Pharmacia, Montreal, Quebec, Canada). The mouse CD4 enhancer was isolated from a mouse phage genomic library by using mouse CD4 cDNA clone (20).

Southern blot analysis. DNA analyses by agarose gel electrophoresis and Southern blotting (36) were done on nylon membranes (Amersham). Probes were ^{32}P labeled with mixed oligonucleotides as primers (11).

Northern (RNA) blot analysis. RNA was isolated by the method of Chomczynski and Sacchi (6). RNA samples were applied to 1% agarose gels containing formaldehyde as described previously (23). Following electrophoresis, RNAs were blotted onto nylon membranes (Amersham). After UV treatment, blots were hybridized with the ^{32}P-labeled 1.8-kb *Eco*R1-*Bam*HI human CD4 cDNA fragment (21) at 65°C and washed in conditions previously described (28).

RNase protection analysis. RNase protection analysis was performed essentially as described by Melton et al. (25). Genomic fragments were cloned in the vector pGEM-3, and 0.5 μg of linearized DNA was used for synthesis of ^{32}P-labeled antisense RNA probe. Two probes were used in this analysis. Probe A consisted of a 0.5-kbp genomic DNA fragment extending upstream from the *Rsa*I site (within exon 3) to the *Nco*I site upstream to exon 2. Probe B consisted of a 0.6-kbp genomic fragment 10 kbp upstream from exon 2, extending from the *Xba*I site through exon 1 to the *Pvu*II site. These antisense ^{32}P-labeled RNA probes were hybridized with total RNA (20 to 30 μg) isolated from CEM and MT-4 $CD4^+$ lymphocytes or from transgenic organs. As a negative control, an equivalent amount of RNA from nontransgenic mouse spleen was used. RNase-protected fragments were fractionated on 8% denaturing polyacrylamide gels (27).

Flow cytometry. The antibodies used in this study, phycoerythrin (PE)-coupled anti-human CD4 antibody Leu3a (Becton Dickinson), fluorescein isothiocyanate (FITC)-coupled antibody anti-murine CD4 GK 1.5), biotin-coupled anti-murine CD8 antibody Ly2, and FITC-coupled anti-murine CD3 antibody 145-2C11, were previously described (27). The cell suspensions obtained from lymphoid organs were stained with antibodies as previously described (27). Detection of biotinylated antibodies was facilitated by using PE-avidin (Southern Biotechnology) as a second-step reagent. Cells were analyzed on a FACScan (Becton Dickinson) as described previously (27).

Sequencing and computer analysis. Sequencing was performed by the dideoxy-chain termination method of Sanger et al. (32). The DNA sequence was determined by isolation and subcloning of specific restriction fragments into either pGEM-3 or pUC-18 vector essentially as described (1). The entire nucleotide sequence of the promoter was determined from both strands, and all sites used for cloning were crossed in the sequencing. [α-^{35}S]dATP (Amersham) and Sequenase (United States Biochemical) were used in sequencing reactions. Sequence alignments and homology and motifs searches were performed with the Genetics Computer Group software package (9).

Construction of transgenes. To construct transgene CD4A/CD4, the 5′ upstream 13-kbp *Sal*I-*Rsa*I fragment of the human CD4 promoter, containing exon 1, intron 1, exon 2, and part of exon 3 up to the *Rsa*I site, was fused to the *Rsa*I site of a 1.7-kbp fragment of the human cDNA sequences derived from plasmid pT4B (21) and a 1.9-kbp *Sal*I-*Eco*RI simian virus 40 region harboring a polyadenylation site. The cloned fragment to be microinjected was isolated as a 16.6-kbp fragment by *Eco*RI and partial *Sal*I digestion. Transgene CD4B/CD4 was constructed by deleting a 4.8-kbp *Sac*I 5′-end fragment (containing exon 1 and part of intron 1) from the transgene CD4A/CD4 (see Fig. 2). The fragment to be microinjected was excised as an 11.8-kbp *Eco*RI fragment. Transgene CD4C/CD4 was constructed by ligating a 1.9-kbp *Eco*RI-*Sal*I fragment containing the mouse CD4 T-cell-specific enhancer (33) to transgene CD4A/CD4. The transgene DNA used for microinjection was excised as a 18.5-kbp *Eco*RI fragment. DNAs, purified by preparative agarose gel electrophoresis, were banded on CsCl as previously described (4, 27).

Production of transgenic mice. One-cell (C57BL/6 × C3H)F_2 embryos were collected, microinjected, and transferred into pseudopregnant CD1 females essentially as described previously (4, 27). The presence of the transgene was confirmed by Southern hybridization with tail DNA, using CD4 cDNA as a probe. Five CD4A/CD4 transgenic founders (5566, 7753, 8263, 8277, and 8786) were produced from 69 pups born. Three CD4B/CD4 (D1, D2, and D9) and four CD4C/CD4 (8918, 8929, 8951, and 8956) transgenic founders were obtained from 11 and 27 pups born, respectively. All founders transmitted the transgene in a Mendelian fashion and appeared phenotypically normal.

RESULTS

Cloning of the human CD4 gene and its flanking sequences. A human placenta genomic library was screened by using the 1.8-kbp cDNA fragment of the human CD4 gene as a probe

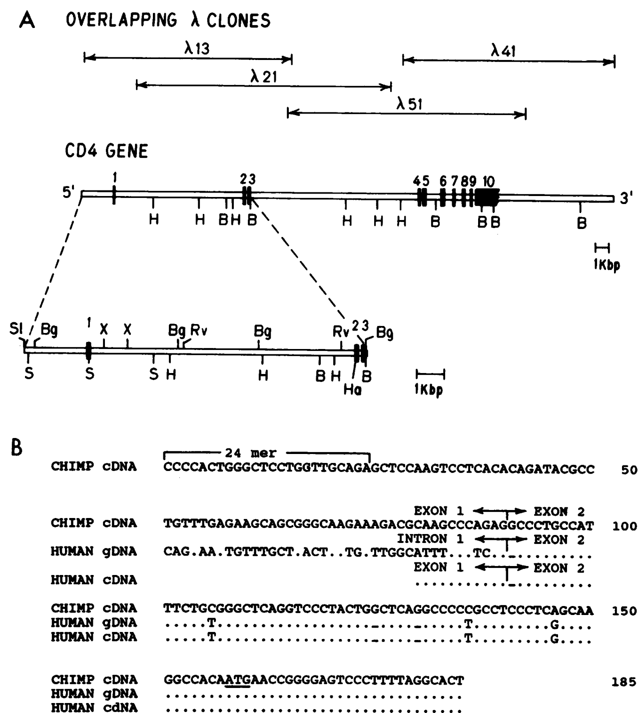

FIG. 1. (A) Structure of the human CD4 gene. A partial restriction map of the gene with exons (black boxes) numbered 1 to 10 and introns (open bar) is shown. The region covered by the four phage clones (λ13, λ21, λ51, and λ41) isolated from the human placenta DNA is indicated by arrows at the top. Restriction endonucleases: B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; Ha, *Hae*III; RV, *Eco*RV; S, *Sac*I; Sl, *Sal*I; X, *Xba*I. The *Sal*I site belongs to the lambda phage vector. (B) Nucleotide sequence alignment of human genomic DNA (gDNA) around exon 2 and the 5' end of the published sequences of the human (21) and chimpanzee (5) CD4 cDNAs. Dots represent nucleotides identical to those in the chimpanzee cDNA, and dashes represent the absence of nucleotides. The initiation codon ATG is underlined. The 24-mer oligonucleotide used to locate exon 1 is indicated.

(21). Several overlapping phage clones were isolated, and four of them, designated λ13, λ21, λ51, and λ41, were selected (Fig. 1A). Restriction analysis and Southern blot hybridization with probes derived from the cDNA revealed that the length of the cloned genomic DNA fragment containing all of the cDNA sequences was 42.2 kbp (Fig. 1A). The DNA inserts of λ13, λ21, λ51, and λ41 were assembled into one large fragment (42.2 kbp) and cloned in the cosmid pWE15. Restriction analysis and Southern hybridization established the colinearity of the cosmid clone with the authentic human CD4 gene (data not shown) and suggested that substantial structural alterations did not occur during cloning of the gene.

To identify the regulatory sequences controlling this gene, we first sequenced the region around its first translated exon. Alignment of this genomic sequence with the nucleotide sequences of previously defined human (21) and chimpanzee (5) cDNA clones, both of which contain part of the 5' untranslated region (Fig. 1B), suggested that the human CD4 gene has an additional exon.

To determine the location of this exon in the human CD4 gene, we synthesized a 24-base oligonucleotide complementary to the 5' end of the chimpanzee cDNA (Fig. 1B) and used it as a probe on the genomic λ13 clone. We were able to locate this exon around the *Sac*I site and at ~10.5 kbp upstream from exon 2 (Fig. 1A). Therefore, in contrast to published results (22), the human CD4 gene was found to comprise 10 rather than 9 exons, interrupted by nine introns of various lengths (~0.13 to 14.2 kbp), similar to the mouse CD4 gene (14).

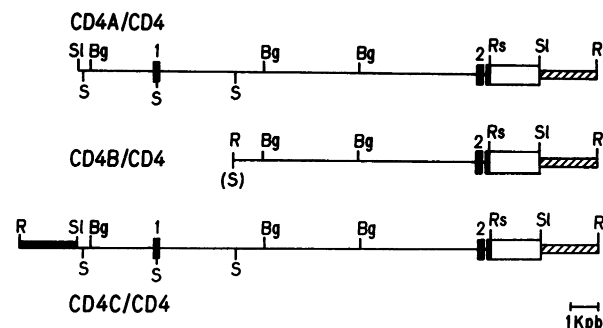

FIG. 2. Structures of the CD4A/CD4, CD4B/CD4, and CD4C/CD4 transgenes. The CD4A/CD4 construct contains 13 kbp of human genomic CD4 sequences (including 2.6 kbp 5' to exon 1), exons 1 and 2, and part of exon 3 up to the *Rsa*I site (black boxes). This fragment was fused to the *Rsa*I site of the human CD4 cDNA (open box) and simian virus 40 fragment containing a polyadenylation signal (hatched box). The CD4B/CD4 transgene was constructed by deleting the 5' *Sac*I fragments from the CD4A/CD4 DNA. The CD4C/CD4 construct contains the entire CD4A/CD4 construct fused to the 1.9-kbp mouse enhancer (black bar). Abbreviations are as defined in the legend to Fig. 1.

Construction of transgenic mice harboring the human CD4 upstream sequences. To test whether the human CD4 upstream sequences are capable of T-cell-specific transcriptional activation in vivo, as well as to identify their *cis*-acting regulatory regions, we constructed three transgenes containing upstream sequences of the CD4 gene linked to the human CD4 cDNA coding sequences as a reporter gene (Fig. 2). The first transgene (CD4A/CD4) contained 12.6 kbp of 5' genomic sequences, including 2.6 kbp of 5' upstream sequences, exons 1 and 2, introns 1 and 2, and part of exon 3 up to the *Rsa*I site. These sequences were fused to the *Rsa*I-*Sal*I human CD4 cDNA fragment derived from plasmid pT4B (21) and to the simian virus 40 fragment containing a polyadenylation signal. Transgene CD4B/CD4 was constructed by deleting 5 kbp of upstream genomic sequences from transgene CD4A/CD4 (Fig. 2), thus deleting the promoter, exon 1, and part of intron 1. Transgene CD4C/CD4 was constructed by adding a 1.9-kbp mouse CD4 enhancer to the CD4A/CD4 transgene. This murine T-cell-specific enhancer located 13 kbp upstream of the first exon of the mouse CD4 gene (33) and its human homolog (3) were identified while our work was in progress. In vitro analysis showed that the enhancing activity of the murine fragment was not restricted to $CD4^+$ T cells but was also observed in $CD4^-$ $CD8^+$ cell lines (33). However, another group was unable to confirm such an enhancer activity in vitro (34). Therefore, we decided to test the activity of this enhancer by in vivo studies. Five, three, and four transgenic founders were produced with the transgenes CD4A/CD4, CD4B/CD4, and CD4C/CD4 DNA, respectively. Lines were derived from each of these founders.

Transgene RNA expression in the CD4A/CD4, CD4B/CD4, and CD4C/CD4 transgenic mice. To determine the expression and tissue distribution of the transgene RNA in these transgenic mice, we used RNase protection and Northern blot

analysis on total RNA isolated from different organs of adult mice. In CD4B/CD4 transgenic mice, low expression was detected by RNase protection with probe A in almost all lymphoid as well as nonlymphoid tissues (data not shown), suggesting the presence of weak promoter activity which is not tissue specific in this transgene and indicating that the sequences conferring tissue-specific transcription to the CD4 gene lie outside the region included in this transgene.

A similar analysis was carried out on tissues of the CD4A/CD4 transgenic mice (Fig. 3A). Four founders (5566, 7753, 8263, and 8277) showed similar patterns of expression, while one founder (8286) did not express the transgene. Transgene RNA expression was the highest in the spleen, lymph nodes, and macrophages. Expression was also found, but to a lesser extent, in the thymus, brain, and intestines and was absent in all other organs tested except the liver and lungs. The weak signal in these latter two organs may reflect the presence of T cells and macrophages, which were found to express the transgene (see below). Similar results were obtained with another probe (probe B) (data not shown). These results indicate that this transgene harbors all sequences necessary to direct expression in macrophages and in lymphoid organs.

Transgene expression in CD4C/CD4 mice was significantly higher than in CD4A/CD4 mice and could be measured by Northern blot analysis. The levels of transgene-encoded transcripts in mice from founder 8951 were highest in the thymus and found to a lesser extent in the spleen and lymph nodes (Fig. 3B). Transgene expression was negative in all other organs tested but detectable in the brain and lungs. The weak signal in these latter two organs may reflect the presence of macrophages, which were found to express the transgene (see below). We also observed weak expression in the intestines, reflecting expression in resident T cells of Peyer's patches (Fig. 3B, lanes 12 and 13) (14a). Similar results were obtained with two other founders (8929 and 8956). These results showed that the CD4C/CD4 transgene contains the regulatory sequences necessary to direct high expression of human CD4 in lymphoid organs.

Expression of the human CD4 cell surface protein in lymphoid cells of CD4A/CD4 and CD4B/CD4 transgenic mice. To measure the levels of expression of the human cell surface CD4 protein on lymphoid cells of transgenic mice, we used flow cytometry with a monoclonal antibody specific to the human CD4 protein. In CD4B/CD4 transgenic mice, no expression of human CD4 was detected in spleen or thymus cells of mice from the three different founder lines (data not shown).

In CD4A/CD4 transgenic mice from founder line 5566, the human CD4 protein was expressed in 11.2% ± 4.2% (n = 7), 17.7% ± 3.6% (n = 8), 28.0% ± 3.3% (n = 5), and 11.8% ± 1.8% (n = 5) of the total cell population of the thymus, spleen, mesenteric lymph node, and circulating mononuclear cells, respectively (Table 1). CD4A/CD4 mice from founders 7753 and 8277 also expressed the human transgene in their lymph nodes and thymuses but at a lower percentage (Table 1).

To determine in which cell populations the human CD4 protein was expressed, we used a two-color immunofluorescence fluorescence-activated cell sorting (FACS) analysis with anti-human CD4 and anti-mouse CD4 or anti-mouse CD8 monoclonal antibodies. In the peripheral lymphoid organs (spleen and lymph nodes) of mice from all three CD4A/CD4 founders, the human CD4 protein was expressed almost exclusively on mouse mature $CD4^+$ $CD8^-$ T cells (over 95%) (Fig. 4B; Table 1) and on very few of mature

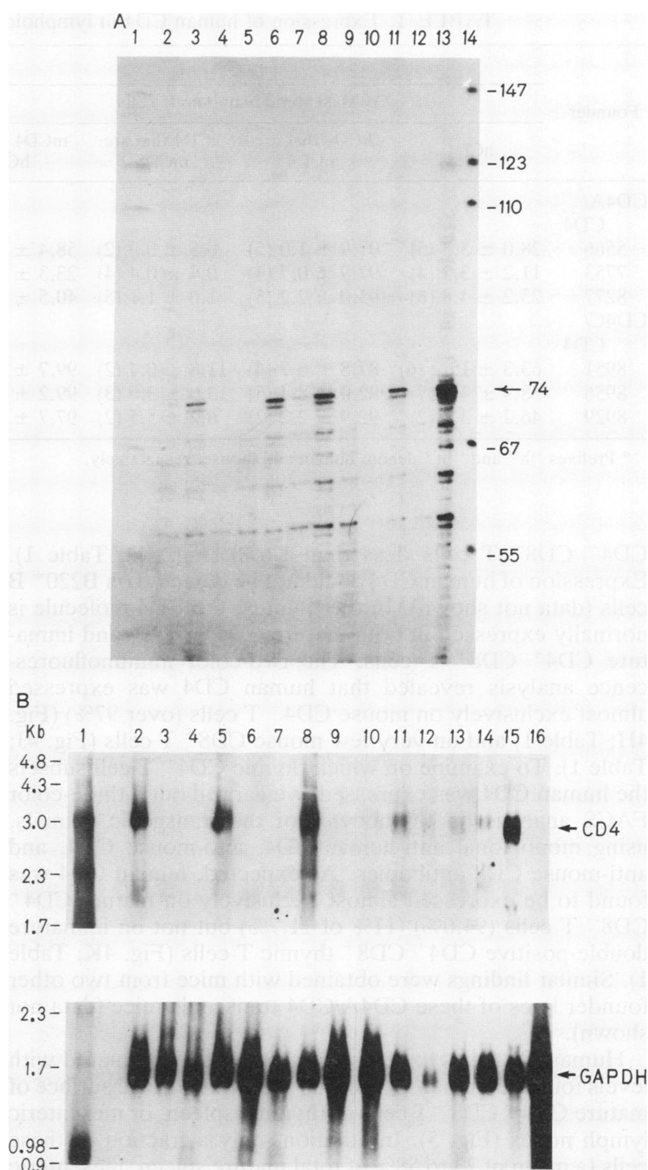

FIG. 3. (A) RNase protection analysis of RNA from different organs of CD4A/CD4 transgenic mice (founder 5566). Total RNAs (20 μg) from different organs were used with ^{32}P-labeled probe A. After digestion with RNases, the fragments were run on an 8% polyacrylamide gel and visualized by autoradiography. A 74-base protected fragment constituting part of exon 3 was taken as a diagnostic fragment for the presence of the transgene. The other protected fragments were nonspecific and appeared also in the negative control. Lanes: 1 and 14, markers; 2 to 11, RNAs from lungs, intestine, kidney, liver, mesenteric lymph node, brain, spleen, thymus, muscle, and macrophages, respectively; 12, RNA from a nontransgenic spleen; 13, RNA from human CEM cells as a positive control. The protected fragment (74 bases) is indicated. By using a densitometer, the relative levels of RNA in lymph node (lane 6), spleen (lane 8), and thymus (lane 9) were calculated to be 4.1, 6.3, and 1.0 respectively. (B) Northern blot analysis of human CD4 RNA extracted from different tissues of a CD4C/CD4 transgenic animal (founder 8951). Lanes: 1, RNA marker; 2, RNA from MT-4 cells (positive control); 3 to 16, RNAs from sciatic nerve, salivary gland, lymph nodes, liver, bone marrow, thymus, heart, kidney, lungs, Peyer's patches, intestine, brain, spleen, and muscle, respectively. The probe used for hybridization was the ^{32}P-labeled human CD4 cDNA. GAPDH, glutaraldehyde 3-phosphate dehydrogenase.

TABLE 1. Expression of human CD4 in lymphoid organs of CD4A/CD4 and CD4C/CD4 transgenic mice[a]

Founder	% ± SE (n)							
	Mesenteric lymph node cells				Thymic cells			
	hCD4	hCD4 that are mCD4	hCD4 that are mCD8	mCD4 that are hCD4	hCD4	hCD4 that are mCD4	hCD4 that are mCD8	mCD4 that are hCD4
CD4A/ CD4								
5566	28.0 ± 3.3 (5)	97.9 ± 1.0 (5)	0.5 ± 0.3 (2)	58.4 ± 12.2 (5)	11.2 ± 4.2 (7)	99.6 ± 0.3 (7)	6.0 ± 1.9 (3)	12.3 ± 5.3 (7)
7753	11.2 ± 3.2 (4)	97.9 ± 0.7 (4)	0.7 ± 0.4 (4)	23.3 ± 6.6 (3)	5.5 ± 2.0 (5)	98.7 ± 0.8 (4)	6.9 ± 1.8 (3)	6.6 ± 2.3 (6)
8277	23.2 ± 4.8 (6)	95.0 ± 2.2 (5)	1.6 ± 1.1 (5)	40.5 ± 7.1 (5)	5.6 ± 1.5 (5)	97.4 ± 1.9 (4)	7.4 ± 2.3 (3)	6.7 ± 1.3 (4)
CD4C/ CD4								
8951	63.3 ± 15.2 (6)	87.8 ± 6.7 (4)	11.8 ± 0.4 (2)	99.7 ± 0.4 (6)	94.7 ± 3.6 (6)	95.9 ± 1.7 (4)	74.3 ± 9.0 (3)	98.9 ± 1.3 (6)
8956	58.9 ± 4.9 (7)	82.0 ± 8.4 (5)	13.0 ± 3.0 (3)	99.2 ± 1.0 (7)	96.2 ± 2.0 (6)	94.2 ± 3.3 (5)	73.9 ± 4.2 (3)	99.5 ± 0.3 (6)
8929	46.2 ± 3.1 (2)	92.9 ± 2.2 (2)	6.2 ± 5.5 (2)	97.7 ± 1.9 (2)	92.8 ± 0.8 (2)	95.7 ± 0.3 (2)	77.7 ± 0.4 (2)	98.8 ± 0.6 (2)

[a] Prefixes "h" and "m" denote human and mouse, respectively.

$CD4^-$ $CD8^+$ T cells (less than 1.6%) (Fig. 4D; Table 1). Expression of human CD4 could not be detected on $B220^+$ B cells (data not shown). In the thymus, the CD4 molecule is normally expressed in both mature $CD4^+$ $CD8^-$ and immature $CD4^+$ $CD8^+$ T cells. The two-color immunofluorescence analysis revealed that human CD4 was expressed almost exclusively on mouse $CD4^+$ T cells (over 97%) (Fig. 4H; Table 1) and on very few mouse $CD8^+$ T cells (Fig. 4J; Table 1). To examine on which thymic $CD4^+$ T-cell subsets the human CD4 was expressed, we carried out a three-color FACS analysis on thymocytes of the transgenic animals, using monoclonal anti-human CD4, anti-mouse CD4, and anti-mouse CD8 antibodies. As expected, human CD4 was found to be expressed almost exclusively on mature $CD4^+$ $CD8^-$ T cells (94.0%) (11% of 11.7%) but not on immature double-positive $CD4^+$ $CD8^+$ thymic T cells (Fig. 4K; Table 1). Similar findings were obtained with mice from two other founder lines of these CD4A/CD4 transgenic mice (data not shown).

Human CD4 was expressed at low levels (compared with levels found in human CEM cells in culture) on the surface of mature $CD4^+$ $CD8^-$ T cells of thymus, spleen, or mesenteric lymph nodes (Fig. 5). In addition, only a fraction of these cells (a mean of 23 to 58% of total mature spleen, mesenteric lymph node, or thymus $CD4^+$ $CD8^-$ T cells, depending on the founder line analyzed) expressed human CD4 (Fig. 4B, E, and K; Table 1). However, after stimulation of splenocytes in vitro with concanavalin A, a T-cell-specific mitogen, the percentage of mouse $CD4^+$ $CD8^-$ T cells expressing the human CD4 molecule increased significantly to 86%, as shown in Fig. 4E and F for a representative mouse of the founder line 5566.

Expression of the transgene was also evaluated on thymocytes and splenocytes of embryos and young mice at different time points after birth. The human CD4 molecule was not expressed in these cells of day 19 embryos or on lymphoid cells of the neonates (data not shown). However, the percentage of mouse T cells expressing human CD4 progressively increased with age both in the thymus and in the spleen (data not shown).

Together, these data clearly show that the CD4A/CD4 transgenic mice express human CD4 in mature $CD4^+$ $CD8^-$ T cells but not in immature $CD4^+$ $CD8^+$ T cells, which represent approximately 80% of thymic T cells. These results suggest that the CD4A/CD4 transgene contains the *cis*-acting elements required to direct expression in $CD4^+$ mature T cells but is lacking those elements necessary for proper expression in immature double-positive T cells. These data also imply that expression of CD4 in mature and immature T cells is controlled by different regulatory elements or alternatively that some regulatory elements control the expression of CD4 at different levels in mature and immature T-cell subsets.

Expression of the human CD4 cell surface protein in lymphoid cells of CD4C/CD4 transgenic mice. To examine the effect of the mouse T-cell-specific enhancer on expression of the human CD4 gene, FACS analyses of cells from the lymphoid organs of mice from four different founders of the CD4C/CD4 transgenic mice were performed. A significant increase in the percentage of human CD4-expressing lymphoid cells of the spleen, compared with that of the CD4A/CD4 transgenic mice, was found (Fig. 5). In mice from founder 8951, human CD4 was expressed in 63.3% ± 15.2% (n = 6) and 94.7% ± 3.6% (n = 6) of the total lymphoid cells of the mesenteric lymph nodes and thymus, respectively (Table 1). Similar results were obtained with the two other CD4C/CD4 founders (8929 and 8956) (Table 1). The fourth founder (8918) expressed CD4 on fewer cells and was not studied further. The intensity of the cell surface expression of human CD4 was also increased significantly in these mice and was comparable to the expression on CEM cells (Fig. 5).

To determine the distribution of human CD4 expression in the lymphoid cell subsets, a two-color FACS analysis was conducted on cells from the mesenteric lymph nodes and thymuses as described above. The analysis indicated that in the mesenteric lymph nodes of all three founders, human CD4 was expressed on the majority (over 87%; founder 8951) of mature $CD4^+$ $CD8^-$ T cells (Fig. 6B; Table 1). In contrast to the CD4A/CD4 transgenic mice, about 11.8% of the mesenteric lymph node cells expressing human CD4 were mature $CD4^-$ $CD8^+$ T cells (Fig. 6D; Table 1). Although almost all (over 97.7%; Table 1) of the mouse $CD4^+$ cells coexpressed human CD4, only a subset (46.8%) of the mouse $CD8^+$ T cells coexpressed it, as shown for a representative mouse in Fig. 6D.

In the thymus, human CD4 was expressed on 95.9% of mouse $CD4^+$ and 74.3% of mouse $CD8^+$ T cells of founder 8951 (Fig. 6I; Table 1). To determine which subsets of thymic $CD4^+$ or $CD8^+$ T cells expressed the transgene, a three-color FACS analysis was performed. This analysis revealed that human CD4 was expressed at high levels on immature $CD4^+$ $CD8^+$ and mature $CD4^+$ $CD8^-$ T cells. On

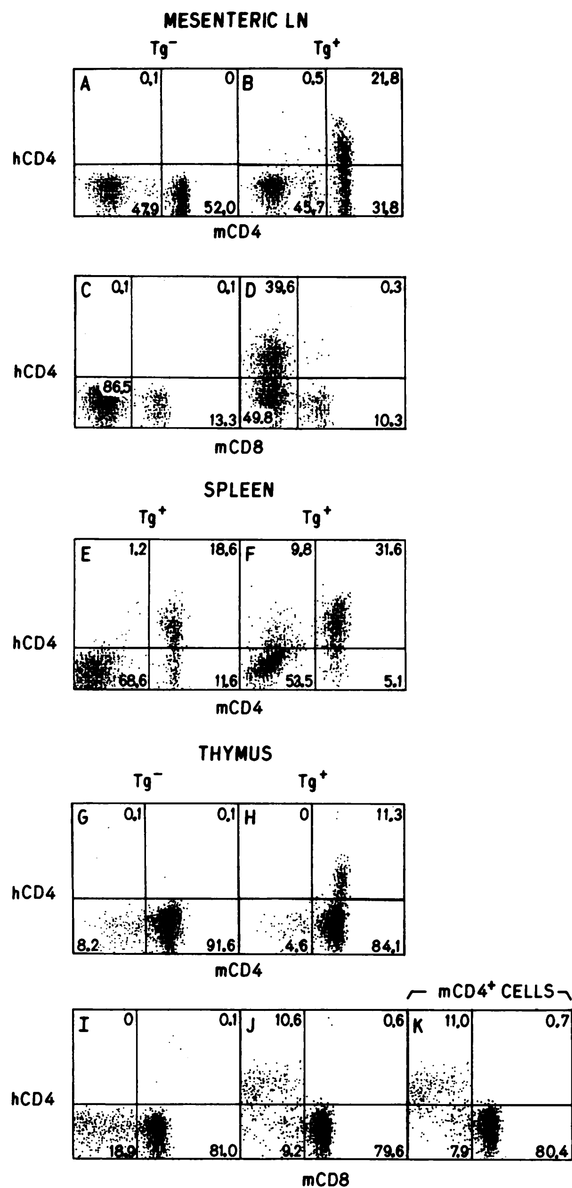

FIG. 4. Detection of human CD4 on lymphocytes of CD4A/CD4 transgenic mice. Cells were harvested from mesenteric lymph nodes (LN), spleens, or thymuses of transgenic (Tg^+) CD4A/CD4 (founder 5566) mice or nontransgenic (Tg^-) littermates and were processed as described in Materials and Methods. In panels A to D, mesenteric lymph node cells of a nontransgenic (A and C) or transgenic (B and D) mouse were doubly labeled with anti-human CD4 monoclonal antibody Leu3A-PE and anti-mouse CD4 (mCD4) monoclonal antibody GK 1.5-FITC (A and B) or with anti-mouse CD8 monoclonal antibody Ly2-FITC (C and D). In panels E and F, spleen cells from a transgenic mouse, before (E) or after (F) stimulation in vitro by concanavalin A for 2 days, were doubly labeled with anti-human CD4 and anti-mouse CD4 antibodies. In panels G to K, thymocytes of nontransgenic (G and I) or transgenic (H, J, and K) mice were labeled with anti-human CD4 monoclonal antibody Leu3A-PE and anti-mouse CD4 monoclonal antibody GK 1.5-FITC (G and H) or with anti-mouse CD8 monoclonal antibody Ly2-biotin-avidin Cy (I and J). In panel K, the cells represented in panel J were labeled in a three-color analysis with monoclonal antibodies anti-human CD4 Leu3A-PE, anti-mouse CD4 GK 1.5-FITC, and anti-mouse CD8 Ly2-biotin-avidin Cy. In this panel, only GK 1.5-positive ($CD4^+$) T cells are represented. Note that most cells expressing the human CD4 molecule are mature $CD4^+$ $CD8^-$ T cells rather than immature $CD4^+$ $CD8^+$ T cells.

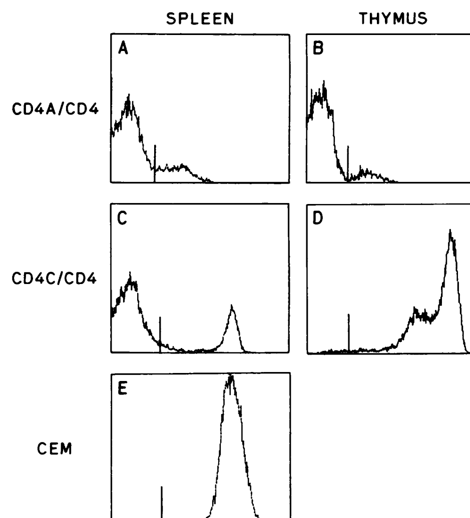

FIG. 5. Comparative levels of expression of human CD4 on human CEM cells and on splenocytes and thymocytes of CD4A/CD4 and CD4C/CD4 transgenic mice. Cells were harvested from the spleens and thymuses of transgenic CD4A/CD4 (founder 5566) (A and B) and CD4C/CD4 (founder 8951) (C and D) mice or were obtained from the established human CEM T-cell line (E). Cells were stained in a direct assay with anti-human CD4 antibody Leu3A-PE. The vertical bar represents the lower limit of positivity determined by direct labeling of the splenocytes or thymocytes of a nontransgenic littermate, using the same antibody.

gated mouse $CD4^+$ thymic cells (Fig. 6J), human CD4 was expressed on 99.9% (75.6% of 75.7%) of the $CD4^+$ $CD8^+$ T cells and on 99.6% (24.2% of 24.3%) of the $CD4^+$ $CD8^-$ T cells. In addition, on gated mouse $CD8^+$ thymic cells (Fig. 6G), human CD4 was expressed on 51.5% (1.7% of 3.3%) of the $CD4^-$ $CD8^+$ thymic T cells.

These results indicate that the mouse T-cell enhancer present in this transgene not only enhanced the levels of expression of the human CD4 surface protein in mature $CD4^+$ subsets but also facilitated its expression on immature double-positive $CD4^+$ $CD8^+$, as well as a subset of $CD4^-$ $CD8^+$ T cells. There was no expression of human CD4 on $CD4^-$ $CD8^-$ thymocytes, indicating that the CD4C/CD4 transgene is capable of driving CD4 expression at the correct stage of thymocyte development. Expression on mature $CD4^-$ $CD8^+$ cells suggests that the CD4C/CD4 construct is still missing negative regulatory element(s) required for totally silencing expression of the CD4 gene in this subset.

Expression of the human CD4 cell surface protein in macrophages of CD4A/CD4 and CD4C/CD4 transgenic mice. In humans, the CD4 protein is expressed not only on lymphoid T cells (19) but also on macrophages (39). However, in contrast to human CD4, mouse CD4 is not expressed on macrophages (7). It was therefore of interest to monitor expression of the human CD4 protein in macrophages of the CD4A/CD4 and CD4C/CD4 transgenic mice. Mouse peritoneal macrophages were collected and doubly stained with anti-human CD4 and macrophage-specific Mac-1 antibodies. Over 33% of the macrophages from the freshly isolated peritoneal cells from founder 5566 of the CD4A/CD4 transgenic mice expressed human CD4 (Fig. 7B). However, after plating on tissue culture dishes and incubation in vitro for 24 h, nearly 100% of adhering macrophages of all tested mice expressed human CD4 (Fig. 7D; Table 2). Similar findings were obtained with mice from founder 8277 of the CD4A/

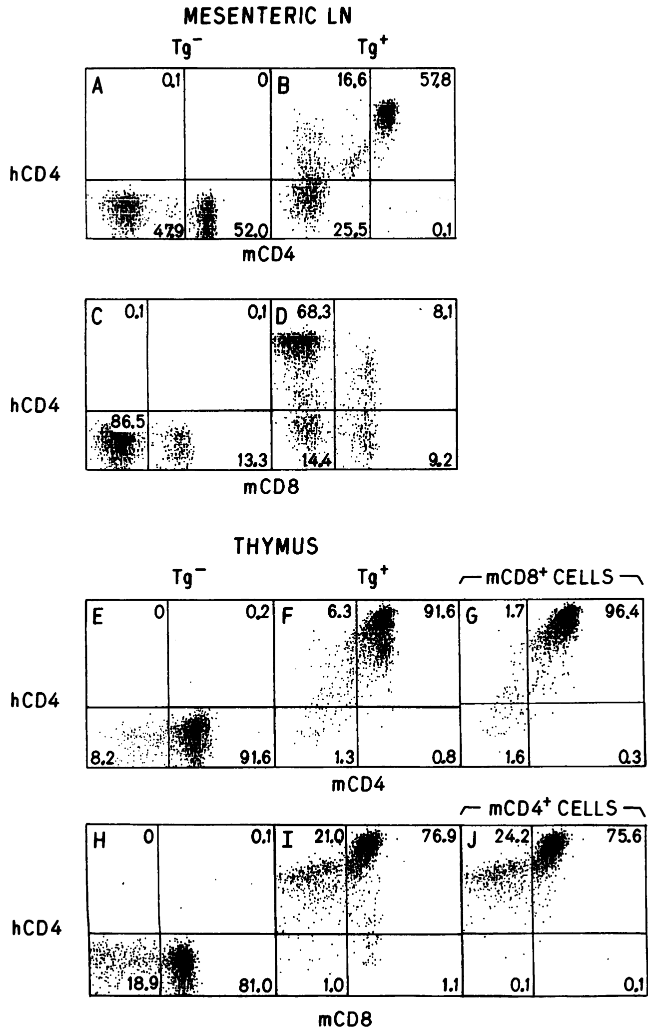

FIG. 6. Detection of human CD4 on lymphocytes of CD4C/CD4 transgenic mice. Cells were harvested from mesenteric lymph nodes (LN) or thymuses of transgenic (Tg^+) CD4C/CD4 mice (founder 8951) or nontransgenic (Tg^-) littermates and were processed as described in Materials and Methods. In panels A to D, mesenteric lymph node cells of a nontransgenic (A and C) or transgenic (B and D) mouse were doubly labeled with anti-human CD4 monoclonal antibody Leu3A-PE and anti-mouse CD4 (mCD4) monoclonal antibody GK 1.5-FITC (A and B) or with anti-mouse CD8 monoclonal antibody Ly2-FITC (C and D). In panels E, F, H, and I, thymocytes of a nontransgenic (E and H) or transgenic (F and I) mouse were doubly labeled with anti-human CD4 monoclonal antibody Leu3A-PE and anti-mouse CD4 monoclonal antibody GK 1.5-FITC (E and F) or with anti-mouse CD8 monoclonal antibody Ly2-biotin-avidin Cy (H and I). In panels G and J, the cells represented in panel F and I were labeled in a three-color analysis with monoclonal antibodies anti-human CD4 Leu3A-PE, anti-mouse CD4 GK 1.5-FITC, and anti-mouse CD8 Ly2-biotin-avidin CY. In panel G, only Ly2-positive ($CD8^+$) T cells are represented; in panel J, only GK 1.5-positive ($CD4^+$) T cells are represented. Note that most mature $CD4^+$ $CD8^-$ and immature $CD4^+$ $CD8^+$ T cells are expressing the human CD4 molecule.

CD4 transgenic mice (Table 2). The same analysis was performed on macrophages of mice from CD4C/CD4 transgenic lines, and macrophage expression of cell surface CD4 was detected in two of three founders tested (Fig. 7D; Table 2) at levels comparable to those found in CD4A/CD4 transgenic mice. This result indicates that the T-cell-specific enhancer has little if any role in expression of human CD4 in macrophages.

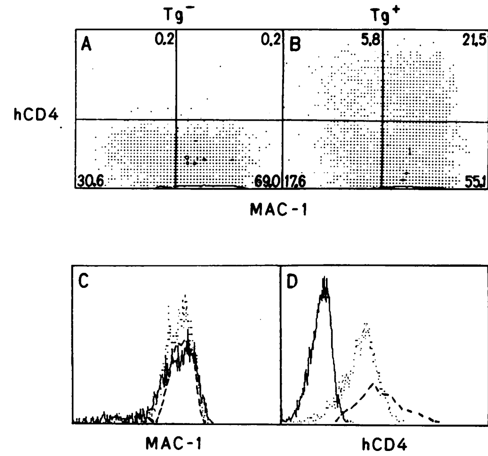

FIG. 7. Detection of human CD4 on macrophages of CD4A/CD4 and CD4C/CD4 transgenic mice. Transgenic CD4A/CD4 (founder 5566) and CD4C/CD4 (founder 8951) mice and a normal littermate were injected intraperitoneally with 1 ml of mineral oil 5 days prior to FACS analysis. Peritoneal cells were harvested and processed as described in Materials and Methods. A portion of these cells was directly labeled (A and B) or plated on tissue culture dishes overnight at 37°C in a CO_2 atmosphere (C and D). In panels A and B, cells were doubly stained with anti-Mac-1 monoclonal antibody–rat anti-goat FITC-coupled antibody (in an indirect assay) and anti-human CD4 (hCD4) monoclonal antibody Leu3A-PE. Panel A represents a nontransgenic (Tg^-) mouse, and panel B represents a transgenic (Tg^+) CD4A/CD4 mouse. In panels C and D, the adherent macrophages were detached from petri dishes and labeled with anti-Mac-1 antibody–rat anti-goat FITC-coupled antibody (C) or with anti-human CD4 monoclonal antibody Leu3A-PE (D). All cytometric analyses were done with a FACScan (Becton Dickinson) apparatus. Symbols: solid lines, nontransgenic mouse; dotted lines, transgenic CD4A/CD4 mouse; dashed lines, transgenic CD4C/CD4 mouse. The slightly higher fluorescence intensity levels seen in the CD4C/CD4 mouse (compared with the CD4A/CD4 mouse) shown in panel D was not seen in every animal analyzed. For most mice studied, these values were comparable between lines CD4A/CD4 and CDC/CD4.

Together, these results show that the 5′ upstream fragment of the human CD4 gene present in the CD4A/CD4

TABLE 2. Detection of human CD4 at the cell surface of plated peritoneal macrophages of CD4A/CD4 and CD4C/CD4 transgenic mice

Founder	No. of mice expressing human CD4/ no. tested[a]
CD4A/CD4	
5566	7/7
7753	1/5
8277	3/3
CD4C/CD4	
8951	4/4
8956	4/4
8929	0/3

[a] Assay done on plated macrophages as described in Materials and Methods. In each positive mouse, all cells expressed human CD4 as shown in Fig. 7D.

transgene contains the *cis*-acting sequences necessary to specifically direct expression of a reporter gene in macrophages.

The expression of human CD4 protein in transgenic mice does not alter the distribution of mouse CD4 and CD8 T cells. Expression of the human CD4 molecule in lymphoid tissues of transgenic mice does not seem to affect the distribution of mouse mature T cells in lymphoid organs (27). To determine whether this was the case in the CD4A/CD4 and CD4C/CD4 transgenic mice, we carried out a FACS analysis on mouse CD4 and CD8 T-cell populations. The sizes of the thymus, spleen, and other lymphoid organs were not significantly different in CD4 transgenic mice compared with normal animals (data not shown). In addition, the patterns of expression of mouse CD4 and CD8 were not significantly altered in these transgenic mice relative to their normal littermates except for a possible modest increase of peripheral $CD8^+$ T cells of the CD4C/CD4 transgenic animal (data not shown). Therefore, the presence of the human CD4 molecule does not appear to significantly affect the distribution of the lymphoid cells in these transgenic mice.

Identification of potential regulatory sequences around exon 1 of the human CD4 gene. To identify potential regulatory sequences within the human CD4 gene, we sequenced the region surrounding exon 1 (Fig. 8A). A computer search analysis revealed the absence of CAAT or TATA box-binding sites and other initiator sequences identified recently (24, 35) but identified a remarkable array of elements and motifs, some of which are characteristic of eukaryotic promoters and regulatory regions found in other genes. For example, the consensus sequence (5'[T/C]AACGG3') which binds to Myb transcription factor was recently found to reside in several locations within the 5' flanking sequences of the mouse CD4 gene and has been postulated to have role in regulation of this gene (34). The sequences also display several unique regions of direct repeats.

Comparative analysis of these human sequences with the homologous mouse sequences revealed six highly conserved regions, indicated by blocks A through F in Fig. 8B. The conserved locations of these sequences in both species suggest a functional role. For example, block E contains the sequence 5'CTTCCTG3', described as an Ets-binding element (also known as the PEA3 binding site) and known to interact with the lymphoid-specific transcription factor Ets-1 (18). However, the locations of many other motifs are not conserved in the human CD4 promoter as in the mouse promoter. For example, the three potential Myb binding sites were found at different locations (underlined in the human promoter) (Fig. 8). It remains to be determined which of these specific motifs play a role in regulation of human CD4 gene expression and how they operate. Recently, in vitro studies have shown that transcription of the human CD4 gene is activated by Ets proteins (30).

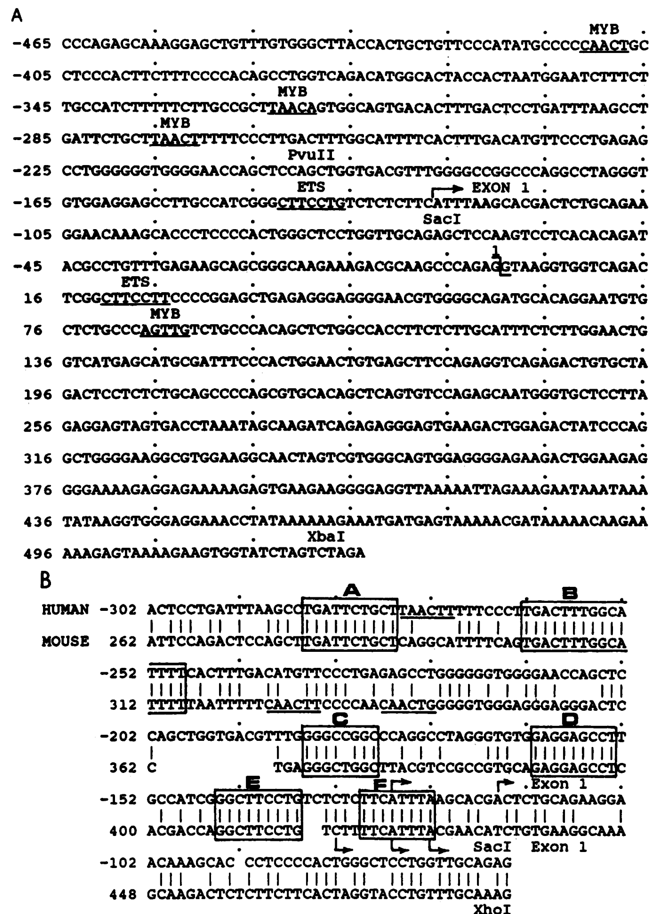

FIG. 8. (A) Nucleotide sequence of the promoter of the human CD4 gene. The sequence upstream of exon 1 is shown. In addition, the sequences of exon 1 and part of intron 1 are presented. The Myb and Ets motifs found in this sequence are underlined. Symbols: ↱ , the major transcription start point localized by RNase protection analysis (30; unpublished data); ⅂ , splice donor site. Reference position 1 is identified as the last nucleotide in exon 1 of the CD4 gene. (B) Conserved sequences in the 5' flanking region of the promoter of the CD4 gene. The 240 bp of the human and mouse sequences when aligned showed six conserved regions, designated blocks A through F. The previously identified Myb motifs are underlined in the mouse sequence. The symbols ↱ and ↳ show the transcription start points of the human (30; unpublished data) and mouse (34) genes, respectively.

DISCUSSION

In this work, we used transgenic mice to study the regulatory elements controlling expression of the human CD4 gene. In humans, the CD4 gene is expressed in macrophages, in $CD4^+$ $CD8^+$ immature T cells, and in $CD4^+$ $CD8^-$ mature T cells. We were able to map some of the elements controlling this cell-specific expression of CD4 within a 12.6-kbp region of the human CD4 gene comprising 2.6 kbp 5' sequences upstream of the transcription initiation site, the first two exons and introns, and part of exon 3. As in human cells, the CD4A/CD4 transgenic mice were able to specifically express the reporter CD4 gene in macrophages and in mature $CD4^+$ $CD8^-$ T cells, indicating that the 5' upstream region of the human CD4 gene, present in the CD4A/CD4 transgene, contains sufficient *cis*-regulatory elements to drive tissue-specific expression in mouse macrophages and mature T cells. Interestingly, Sands and Nikolic-Zugic (31) have recently identified several possible transcriptional control elements, defined as DNase I-hypersensitive (DH) sites, within the murine CD4 gene. Seven of these DH sites were found to be T cell specific, and five of them were located within the 12-kb region around the first intron of the murine CD4 gene. The analogous human region was used in our transgene CD4A/CD4 and probably contains similar T-cell-specific DH sites. The exclusive expression of human CD4 in mature $CD4^+$ $CD8^-$ T cells of the CD4A/CD4 transgenic mice is also in agreement with the previously

reported in vitro analysis of the mouse CD4 promoter (34). Sui et al. (34) found that a 172-bp fragment of the mouse CD4 promoter directed the expression of a luciferase reporter exclusively in mature $CD4^+$ $CD8^-$ T-cell lines, not in mature $CD4^-$ $CD8^+$ or immature $CD4^+$ $CD8^+$ T-cell lines. This 172-bp fragment contains one of the T-cell-specific DH sites identified by Sands and Nikolic-Zugic in $CD4^+$ $CD8^-$ cell lines but not in $CD4^-$ $CD8^+$ cell lines (31).

The expression of human CD4 in macrophages is of particular interest since the mouse CD4 gene itself is not expressed in macrophages. Therefore, our data indicate that mouse macrophages contain all factors necessary for transcribing the human CD4 gene. Our results also suggest that the endogenous mouse CD4 gene fails to be expressed in mouse macrophages because it either lacks the required macrophage-specific regulatory elements or contains suppressor sequences inhibiting expression. The locations of the regulatory sequences allowing expression in mature $CD4^+$ $CD8^-$ T cells and in macrophages are not precisely known and could be anywhere within the 12.6-kbp fragment used in front of the reporter gene. Our data do not allow us to localize them with more precision. However, the in vitro findings of Siu et al. (34) indicate that they may reside within the 2.6 kbp of sequences upstream of the first exon. We have sequenced part of this region and found that it is highly homologous to the mouse 172-bp sequences previously identified (34) and harbors a remarkable number of motifs previously shown to function as regulatory elements in other genes (12, 26). A more refined analysis of this region will be required to identify which individual elements are required for tissue-specific expression in $CD4^+$ $CD8^-$ T cells and in macrophages.

Although a 12.6-kbp fragment of the CD4 gene was sufficient to express the reporter CD4 cDNA gene in macrophages and in mature $CD4^+$ $CD8^-$ T cells, it was not sufficient to support its expression in immature $CD4^+$ $CD8^+$ T cells, indicating that regulatory elements specific for $CD4^+$ $CD8^+$ T cells are still missing in the CD4A/CD4 construct. This result also suggests that for control of CD4 gene expression, these immature $CD4^+$ $CD8^+$ T cells use a different set of regulatory elements than mature $CD4^+$ $CD8^-$ T cells do, as previously suggested (3). This conclusion was confirmed by the results obtained with the CD4C/CD4 transgenic mice, which expressed their transgene not only in mature $CD4^+$ $CD8^-$ T cells but also in immature $CD4^+$ $CD8^+$ T cells. The transgene carried by these mice contains an additional 1.9-kbp mouse fragment harboring a T-cell-specific enhancer identified by Sawada and Littman (33). The different control of human CD4 gene expression in mature and immature $CD4^+$ T cells observed in our transgenic mice confirmed earlier work done in vitro by transient transfection techniques (34) and in transgenic mice (3). This finding was also corroborated by our previous study of a mouse mammary tumor virus (MMTV) promoter ($MMTV^D$) in transgenic mice (27). We found that the $MMTV^D$ promoter was able to drive expression of surrogate genes specifically in immature $CD4^+$ $CD8^+$ T cells but not in mature $CD4^+$ $CD8^-$ T cells. Together, these results suggest that the regulatory elements present in the $MMTV^D$ long terminal repeat or around the CD4 gene are recognized differently in mature and immature T cells, as seems the case in our CD4A/CD4 and CD4C/CD4 transgenic mice.

Expression of the human CD4 protein detected in murine macrophages of CD4C/CD4 transgenic mice was at about the same levels as expression found in macrophages of CD4A/CD4 transgenic mice, suggesting that the T-cell-specific enhancer has little if any effect on levels of expression in macrophages. However, levels of human CD4 expression in murine mature $CD4^+$ $CD8^-$ T cells of the CD4C/CD4 transgenic mice were much higher than those in the CD4A/CD4 transgenic animals, approaching the levels found in human $CD4^+$ T cells, confirming the enhancing activity of this 1.9-kbp fragment (33). The expression of CD4 in immature $CD4^+$ $CD8^+$ T cells suggest that this 1.9-kbp mouse enhancer fragment harbors *cis*-acting elements required for activating human CD4 expression in this T-cell subset. However, the CD4C/CD4 transgene was also expressed inappropriately in $CD4^-$ $CD8^+$ T cells, indicating that this construct is still lacking the *cis*-acting element required to silence expression of the human CD4 transgene in these mature $CD8^+$ T cells emerging from their progenitors, the $CD4^+$ $CD8^+$ T cells. Such control is not without precedent, since a silencer element outside the TcR-α enhancer was shown to inactivate the enhancer effect in TcR-$\gamma\delta$ T cells and in non-T cells but not in TcR-$\alpha\beta$ T cells (38). Therefore, additional *cis*-acting elements may be required to inactivate the expression of CD4 in $CD4^-$ $CD8^+$ T-cell subsets.

While this work was in progress, three other groups (3, 13, 16) attempted to achieve the appropriate expression of human CD4 in transgenic mice by using CD4 genomic constructs. Gillespie et al. (13) used a construct very similar to our CD4C/CD4 transgene and reported expression in the $CD4^+$ subsets of peripheral blood mononuclear cells. Our results seem to confirm their data. However, since no other cell surface marker was used and since a detailed FACS analysis of the cells expressing human CD4 in the thymus was not carried out, it is difficult to further compare their results with ours. On the other hand, it appears that our data are significantly different from the recent results of the other two groups (3, 16), who used genomic human CD4 transgenes containing all of the exons. In contrast to the $CD4^+$ T-cell-specific expression observed in our CD4A/CD4 transgenic mice, both groups (3, 16) failed to demonstrate any expression of the human CD4 on mouse $CD4^+$ thymocytes of their mice harboring a transgene lacking the CD4 enhancer. One group (3) observed expression in the thymus of these mice, but it was in non-T cells. With respect to the peripheral tissues of the same mice harboring the enhancerless transgene, the results of the two groups diverged. Killeen et al. (16) found no expression of human CD4 in spleen T cells, while Blum et al. (3) obtained a low level of expression in the spleen (4% of $CD4^+$ splenocytes), but most of the human CD4 expression was in B cells. In contrast to these results, we have observed, with the enhancerless CD4A/CD4 transgene, significantly higher levels of expression of human CD4 (in 11 and 18% of the T-cell populations of the thymus and spleen, respectively), and we have not been able to detect expression of human CD4 in B cells of any of our transgenic mice. The basis for these discrepancies is not clear but may be related to the exclusive use of genomic sequences, and specifically the 3′-end CD4 sequences, by these groups. The addition of the human T-cell-specific enhancer to the human genomic CD4 transgene constructed by Blum et al. (3) significantly increased the expression of CD4 in $CD4^+$ $CD8^-$ and $CD4^+$ $CD8^+$ T cells and in a small population of $CD4^-$ $CD8^+$ thymocytes and led to an increased number of spleen $CD4^+$ $CD8^-$ T cells coexpressing human CD4 (11% versus 4% in the enhancerless transgene). These results are in agreement with our data for the CD4C/CD4 trangenic mice, although the enhancing effect in the $CD4^+$ T splenocytes was smaller than the effect that we observed. Similarly, Killeen et al. (16) found that

addition of the murine T-cell-specific enhancer to the human genomic CD4 construct led to an increased expression of CD4 specifically in peripheral $CD4^+$ T cells, as we have also observed. However, these investigators (16) did not observe expression of human CD4 in mature $CD8^+$ T cells, as we did in CD4C/CD4 transgenic mice.

Together, our results and those of others (3, 13, 16) indicate that expression of the CD4 gene is controlled very similarly in mouse and human cells. This complex expression system in different cell types seems to be controlled by several regulatory elements which appear to be distinct, as previously suggested (3). Some of these elements control expression in mature $CD4^+$ $CD8^-$ T cells, others (such as the T-cell-specific enhancer) allow expression in immature $CD4^+$ $CD8^+$ T cells, some others direct expression in macrophages, and finally others seem to be involved in the down regulation of the CD4 gene in $CD4^-$ $CD8^+$ cytotoxic T cells. Alternatively, one can hypothesize that the same regulatory elements of the human CD4 gene control expression at different levels in different subsets of T cells. Some of the conflicting results obtained by different groups may indicate that the context in which these various elements are assembled may be of great importance. A more refined analysis leading to the identification of each of these elements should explain some of these discrepancies.

Finally, these transgenic mice, expressing human CD4 in a tissue-specific manner, may be instrumental in understanding some aspects of HIV pathogenesis, since CD4 appears to be the main receptor for HIV.

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