CD4 and CD8 expressions in African green monkey helper T lymphocytes: implication for resistance to SIV infection

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Abstract

We found that most peripheral CD4 cells co-express a low density of CD8α antigen in African green monkeys (AGM). Further, the cell surface expression of CD4 and the expression of CD4 mRNA underwent a decrease when purified CD4CD8low cells were cultured with mitogen and IL-2. These observations suggest that AGM CD4 cells are subject to loss of CD4 expression after lymphocyte activation. Part of the peripheral CD8 fraction exhibited a significant helper activity which suggested the phenotypic conversion in helper T cells from CD4+ to CD4− in vivo. Simian immunodeficiency virus (SIV) grew well in CD4 panning cells following SIV infection. In contrast, CD4CD8low cells were resistant to SIV infection after their conversion to CD4− cells.

Introduction

Natural infection with simian immunodeficiency virus (SIV) is known to occur in African green monkeys (Cercopithecus aethiops; AGM) (1,2). Although infectious SIVagm has been isolated from this species, the actual onset of the disease has not been recognized even in experimental animals inoculated with SIVagm (3–5). The functional immune response of AGM to SIVagm is very similar to the response of humans to human immunodeficiency virus (HIV) (6). For example, neutralizing antibodies were found to be low or absent in the sera of infected AGM. Thus, an AGM-specific vigorous immune response that keeps the AGM healthy after SIV infection has not been identified. The degree of sequence variation of SIVagm in vivo is reduced compared to that of HIV-1 in humans (7). The in vivo viral load in healthy AGM remains relatively low (8), and the reasons for the limitation of variability and virus load are still unknown. Several factors released from CD8 cells that suppress HIV replication have been described. Among such factors, IL-16 derived from AGM could more effectively inhibit the HIV-1 replication than human IL-16 (9). IL-16 represses HIV-1 promoter activity (10). In fact, CD8 cells play an important role in the host response to HIV/SIV infection by antigen-specific cytotoxic activity as well as in the production of soluble inhibitors. From a different standpoint, we studied the mechanism by which AGM can escape from the attack of SIVagm. We report here the distinct characteristics of helper T lymphocytes in this species.

Methods

Flow cytometry

Peripheral blood mononuclear cells (PBMC) were separated by the Ficoll (Pharmacia, Uppsala, Sweden) centrifugation method. The cells were stained with CD4 [Leu3a and phycoerythrin (PE–Leu3a)] and CD8α [FITC–, PE– and peridinin chlorophyll protein (PerCP–Leu2a)] mAb (both from Becton Dickinson), CD16 (PE– and PE–Cy5-3G8; PharMingen, San Diego, CA), CD20 (PE–Leu16; Becton Dickinson), CD25 (FITC–IL-2 receptor α; Becton Dickinson), CD27 (FITC–LT-27; Monosan, Uden, The Netherlands), CD122 (FITC–Mikβ-1; Nichirei, Tokyo, Japan), FITC–HLA-DR (Becton Dickinson), monkey CD3 (FITC–FN18; Biosource International, Camarillo, CA), and our original anti-simian U series of mAb such as FITC–U2 (CD8) (11), PE–
U7b (CD4) and FITC–U4 (mature T cells) (11) were also used. The distribution of T Cell repertoires was further analyzed by three-color flow cytometry with PE–Leu3a, PerCP–Leu2a and FITC–V9 antibodies (T Cell Sciences, Boston, MA). After washing, the samples were fixed and analyzed using a FACScan (Becton Dickinson). The thymuses of SIV-uninfected AGM aged 2 years old were minced in the medium [RPMI 1640 (Gibco/BRL, Grand Island, NY)] 15% fetal bovine serum] and then ground through a mesh screen. Thymocytes were stained by the same procedures as those used for peripheral lymphocytes.

We also examined the intracellular expression of CD4 antigen in the cultured CD4 cells. Purified CD4 (Leu3a) antibody was added for blocking cell surface CD4 antigen. After washing and fixation, the samples were incubated with PE–Leu3a antibody in a permeabilization buffer (Caltag, South San Francisco, CA) for 15 min at room temperature. After washing, the intracellular expression of CD4 antigen in cultured CD4 cells was analyzed by flow cytometry.

Cell sorting and culture
AGM PBMC were stained with PE–Leu3a, FITC–Leu2a and PE–Cy5-3G8 antibodies, and then peripheral CD4CD8low cells were sorted out aseptically with an EPICS Elite ESP cell sorter (Coulter, Hialeah, FL). The purity was usually >99.8%. The sorted cells were cultured in the medium containing 5 μg/ml of concanavalin A (Con A, Pharmacia) and 100 U/ml of human recombinant IL-2 (Shionogi, Osaka, Japan) at 37°C in a humidified 5% CO2 atmosphere. The CD4 and CD8 expression of the cultured cells was analyzed at the points of cultivation indicated in Fig. 4(d–f). The scatter gates were set on the blast cell fraction.

**RT-PCR**
To confirm that the loss of CD4 expression was due to the regulation in transcription, the expression of CD4 mRNA was investigated by RT-PCR. The expressions of CD8α and CD8β mRNA were also examined. The total RNA was isolated from peripheral CD4CD8low cells, cultured CD4 cells and cultured CD8bright cells by the acid guanidinium thiocyanate–phenol–chloroform method (12). Total RNA (50 ng) was exposed to an antisense primer, RNase inhibitor, RT (both from Takara, Shiga, Japan) and 1×RT buffer for 15 min at 42°C. In the semiquantitative analysis, dilutions (1:3) of cDNA in water were then combined with a sense primer, 200 μM dNTP and 1×PCR buffer. The resultant solution was subjected to 30 cycles of incubation, with each cycle consisting of denaturation for 1 min at 94°C, annealing for 2 min at 55°C (CD4) or 60°C (CD8) and extension for 2 min at 72°C. Each sample was next subjected to 2% agarose gel electrophoresis and subsequent ethidium bromide staining. β-Actin mRNA was also amplified in each sample as an internal control. The primers designed on the basis of human nucleotide sequences (13–16) are listed in Table 1. The PCR products of the CD4, CD8α and CD8β genes were cloned and sequenced by dideoxy termination methods to estimate the homology of the nucleotide sequences between man and AGM.

### Table 1. The primer sequences used in this study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4-F</td>
<td>5'-TGGGACCTGGACATGCAC-3'</td>
<td>231</td>
</tr>
<tr>
<td>CD4-R</td>
<td>5'-GCCTGGTCCAGCCAAG-3'</td>
<td>441</td>
</tr>
<tr>
<td>CD8α-F</td>
<td>5'- TGCCGCGAGCGACGC-3'</td>
<td>441</td>
</tr>
<tr>
<td>CD8β-F</td>
<td>5'- TGGGACCTGGACATGCAC-3'</td>
<td>432</td>
</tr>
<tr>
<td>CD8β-R</td>
<td>5'- TGGGACCTGGACATGCAC-3'</td>
<td>661</td>
</tr>
</tbody>
</table>

**Assay of helper activity**
B cells were prepared from PBMC of uninfected monkeys by the nylon wool column method. Adherent cells were used as the B cell fraction. Surface Ig were detected in >95% of the adherent cells by flow cytometry. CD4CD8low, CD16 CD8low, CD16 CD8bright cells were sorted out aseptically from PBMC. The purity of each cell fraction used was >99.8%. The numbers of cells of each fraction indicated in Table 3 were added to 2×105 autologous B cells. The mixtures were cultured in a 96-well U-bottom plate in a 200 μl volume of medium containing 2 μl of pokeweed mitogen (PWM, Gibco) for 1 week at 37°C in a humidified 5% CO2 atmosphere. Adherent cells alone were also cultured as a negative control. The IgG released in the culture supernatant was estimated by an indirect ELISA. The values represent the mean of two wells.

**SIV infection in vitro**
The target cells were prepared by two different methods. (a) In the first method, 106 PBMC from an SIV-seronegative monkey was infected with SIVagmTYO-1D (105 TCID50) as measured by the induction of giant-cell formation in a human CD4 cell line, MOLT4 clone 8) in 1 ml volume of medium at 37°C for 1 h. SIVagmTYO-1D propagates in AGM PBMC more efficiently as compared to the original isolate, SIVagmTYO-1 (17). After being washed in PBS, the sample was stained with the CD4 (Leu3a) antibody at 4°C for 1 h. Following further washing, CD8+ cells were also used as target cells. First, CD4 CD8low, CD16–CD8low, CD16–CD8bright cells by the acid guanidinium thiocyanate–phenol–chloroform method (12).

**Table 1. The primer sequences used in this study.**
RT assay

Culture supernatants were centrifuged to pellet-down virus particles and the resultant pellets were dissolved in a TNE buffer containing 1% NP-40 (BDH, Poole, UK) for 30 min on ice. Then, 100 µl of a reaction mixture containing poly(A), oligo(dT), MgCl₂ and ³²P-labeled deoxythymidine 5’-triphosphate (dTTP) was added, and the materials were incubated for 1 h at 37°C. An aliquot of 50 µl of the mixture was spotted onto DE81 paper, air-dried, washed five times in phosphate buffer and then one additional time in 95% ethanol. The paper was subsequently dried and exposed to X-ray film for autoradiography.

Results

Characteristics of AGM lymphocytes

Figure 1(C) shows a two-color flow cytometric profile of peripheral blood lymphocytes (PBL) stained with CD4 (Leu3a) and CD8α (Leu2a) antibodies. CD8 cells formed the major subset in AGM (72.0 ± 8.6%, average ± SD, n = 13) and CD4 cells (19.8 ± 6.0%) shared a low density of the CD8α antigen. CD4 single-positive cells were barely detected (1.5 ± 0.9%). A similar result was obtained by using antisimian CD4 (U7b) and CD8 (U2) antibodies (Fig. 1F). CD20 reacted with 6.2 ± 3.3% of PBL. The number of peripheral B cells was relatively low compared to those of man and other species of monkeys. A whole blood lysis technique for cell staining gave similar results, which indicated that Ficoll-diathizolate purification did not result in a loss of CD4 cells. No significant difference was recognized among monkeys which were both negative and positive for the SIVagm antibody.

CD4 cells expressed the T cell markers such as CD3 and U4 antigens (Fig. 2C and E) and all T cells were CD8α positive (Fig. 2D and F). Figure 3 shows the result of RT-PCR amplification of each message derived from the freshly isolated CD4CD8low cells. PCR products with the predicted size (231 and 441 bp) could be identified by the use of CD4 or CD8α gene-specific primers. In addition to CD4 transcripts, the expression of CD8α mRNA was also recognized in the peripheral CD4+ cells. No PCR product was detected using CD8β primers. These results demonstrated that the peripheral CD4 cells co-expressed CD8α antigen.

CD4CD8low cells lacked activation markers such as IL-2 receptors [2.5 ± 1.6% positive for α chain (n = 4), 0.4 ± 0.4% positive for β chain] and MHC class II molecules (3.6 ± 2.6%). On the contrary, the expression of a marker for naive T cells was significantly higher in CD4CD8low cells than those in CD8low cells. Most of CD4CD8low cells expressed CD27 molecules (94.6 ± 1.9%, n = 6) whereas only part of CD8low cells shared this antigen (59.4 ± 15.1%, P < 0.001, t-test). It has been suggested that the CD27+ population in human CD4 cells contains primed T cells which evolve in vivo from extensive cellular division (19). These findings, taken together, suggest that the majority of AGM CD4CD8low cells are non-activated, naïve T cells. Table 2 shows the distribution of T cell repertoire detected by anti-V₆ and -V₈ antibodies in CD4CD8low, CD8low and CD8bright subsets. No significant difference of the repertoire distribution was recognized among T cell subsets in this study.

The expression of CD4 and CD8 in thymocytes from AGM was also investigated. CD4 single-positive cells represented <2% of the thymocytes as observed in PBL (Fig. 11). In addition to CD4CD8bright cells distributed in b in the figure, CD4CD8low cells (region a) were also recognized in the AGM thymocytes.

CD4/CD8 expression after stimulation

Peripheral CD4CD8low cells were highly purified by sorting and cultured with Con A and IL-2 in an attempt to establish a CD4 cell line from the AGM. Figure 4(I) indicates the result of a purity check of freshly isolated CD4CD8low cells. The positive percentage of CD4 cells was >99.9% in this sample and no contamination of peripheral CD8 cells was recognized. Surprisingly, the cell surface CD4 expression decreased after stimulation and most proliferating cells lacked detectable CD4 molecules within 12 days (Fig. 4J–L). Figure 5 indicates the expression of intracellular CD4 antigen in the cultured CD4 cells at 12 days of cultivation. The down-regulation of CD4 was not accompanied by internalization or modulation of surface CD4 molecules since no detectable expression of intracellular CD4 antigen was recognized.

Figure 6 indicates the results of semiquantitative analysis of each message derived from the cultured CD4 cells. CD4 mRNA could be identified at 4 days of cultivation; however, the expression decreased gradually and almost no mRNA could be detected at 12 days of cultivation. To confirm whether or not this PCR product was derived from the AGM CD4 transcripts, the nucleotide sequence of the PCR product was determined. The homology of the sequences between man and the monkey was ~97% as previously reported (20). These results demonstrated that lymphocyte activation induced the down-regulation of CD4 mRNA expression, resulting in the loss of surface expression of the CD4 molecule.

The conserved sequences between the human CD8 genes and the PCR products with sizes of 441 bp (95% identical to the CD8α sequence) or 432 bp (96% identical to the CD8β sequence) were also recognized. Therefore, it was confirmed that these PCR products were derived from the AGM CD8α and CD8β transcripts respectively. CD8β mRNA expression was not detected in cultured CD4 cells, in contrast to the cultured CD8 cells derived from peripheral CD8bright lymphocytes used as a control for CD8β gene expression.

Helper activity of CD8 cells

The loss of CD4 molecules was also identified when AGM lymphocytes were stimulated by super antigens such as staphylococcal enterotoxin B (SEB) or SED (data not shown). These observations suggest that AGM CD4+ cells are subject to loss of CD4 expression after antigen stimulation. If CD4CD8low cells lose their CD4 expression after stimulation in AGM, CD8 helper T cells must develop in the periphery. To confirm this, CD4CD8low, CD8low and CD8bright cells were purified and the helper activity of each cell fraction was investigated. Natural killer cells with CD8 were gated out by the CD16 antibody. The proportion of CD4CD8low cells in the CD16+ lymphocytes was 23–27% and that of CD8low cells was 18–25%. Table 3 shows the helper activities in PWM-
induced IgG production. CD4CD8\textsuperscript{low} cells were functional with a normal helper activity whereas CD8\textsuperscript{bright} cells lacked this activity. In AGM, CD8\textsuperscript{low} cells also displayed significant helper activity, but the levels were relatively low compared to those of the CD4CD8\textsuperscript{low} cells. CD8\textsuperscript{low} cells demonstrated an equivalent level of helper activity to that of CD4CD8\textsuperscript{low} cells when twice the cell number of CD4CD8\textsuperscript{low} cells was added to the culture.

**SIV growth in CD4 cells**

These findings are very interesting in relation to the defensive mechanism of AGM against SIV infection. The growth of SIV\textsubscript{amgTYO-1D} in CD4 cells was determined by RT assay. CD4 cells were isolated by CD4 panning after SIV infection. SIV grew well in this cell fraction (Fig. 7a) and a cytopathic effect (CPE), such as giant-cell formation, was induced after 3 days of culture. In contrast, the SIV growth was very low in cells converted from being CD4\textsuperscript{+} to CD4\textsuperscript{−} cells following cultivation for 1 week prior to SIV infection (Fig. 7b). No CPE was recognized in this culture. The proliferation rates of cultured cells in experiments (a) and (b) were almost equal. The cell numbers in experiments (a) and (b) were in the ratio 1:1.1 at 7 days and 1:0.95 at 14 days of cultivation. Therefore, the lack of virus growth in experiment (b) was not due to the poor growth of pre-cultured cells.

In addition, we prepared the standard culture with
CD4 and CD8 expression in monkey helper T lymphocytes

Fig. 2. CD3 and U4 expression in AGM CD4 and CD8 cells. (A) FSC versus SSC dot blot. (B) Negative control cells stained with control PE- and FITC-IgG antibodies. (C) AGM PBL stained with CD4 (PE–Leu3a) and CD3 (FITC–FN18). CD4⁺CD3⁻ cells, <1% and CD4⁺CD3⁺ cells, 21%. (D) AGM PBL stained with CD8 (PE–Leu2a) and CD3 (FITC–FN18). CD8⁺CD3⁻ cells, 14% and CD8⁺CD3⁺ cells, 80%. (E) AGM PBL stained with CD4 (PE–Leu3a) and FITC–U4. CD4⁺U4⁻ cells, <1% and CD4⁺U4⁺ cells, 20%. (F) AGM PBL stained with CD8 (PE–Leu2a) and FITC–U4. CD8⁺U4⁻ cells, 14% and CD8⁺U4⁺ cells, 80%.

SIVmac251 and pre-cultured CD4 cells from a rhesus monkey. In this species, the expression of the CD4 molecule had been maintained throughout the culture. SIV grew well as observed in experiment (a) in this control culture (Fig. 7c). These results, taken together, suggest that the CD4 molecule may be the principal receptor for SIVagmTYO-1D in AGM. CD4 masking of CD4CD8low cells can explain the finding of insufficient growth of the virus (1) and a lack of CPE (15) in cultured cells.

Discussion

The peripheral CD4CD8low cells of AGM are non-activated mature T cells which differ from activated T cells (21,22). Although peripheral CD4CD8low cells have been detected in patients with certain diseases (23–25), no signs and symptoms of disease were recognized in the monkeys which we examined.

Mitogen stimulation exerts no effect on the down-modulation of CD4 molecules of human lymphocytes (26). In fact, we confirmed when human peripheral CD4 cells were highly purified and cultured in the same condition as described in this paper, the expression of the CD4 molecule and CD4 mRNA had been maintained throughout the culture. Therefore, our finding of the loss of CD4 expression in AGM does not reflect an experimental artifact.

The loss of CD4 molecules in purified CD4CD8low cells was confirmed by employing other mAb which recognize different epitopes from Leu3a such as NuTmH/I (Nichirei, Tokyo, Japan) and our original simian CD4 antibody, termed U7b (data not shown). We also verified that CD4 (NuTmH) antibody immunoprecipitated a 55–60 kDa molecule from AGM PBL.

Table 2. TCR repertoires in each subset (data from three monkeys are summarized)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Percent in CD4CD8low cells</th>
<th>Percent in CD8low cells</th>
<th>Percent in CD8bright cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>βV5(a)</td>
<td>1.5, 0.8, 1.3</td>
<td>0.8, 1.5, 1.7</td>
<td>1.6, 0.9, 2.2</td>
</tr>
<tr>
<td>βV5(b)</td>
<td>0.0, 0.0, 0.0</td>
<td>0.0, 0.0, 0.4</td>
<td>0.0, 0.0, 0.1</td>
</tr>
<tr>
<td>βV5(c)</td>
<td>0.0, 0.0, 0.7</td>
<td>0.7, 2.1, 4.7</td>
<td>0.2, 0.1, 0.7</td>
</tr>
<tr>
<td>βV8(a)</td>
<td>0.0, 0.0, 0.0</td>
<td>0.6, 0.2, 0.5</td>
<td>0.1, 0.0, 0.0</td>
</tr>
<tr>
<td>βV12(a)</td>
<td>0.0, 0.0, 0.0</td>
<td>0.4, 0.3, 0.4</td>
<td>0.0, 0.1, 0.2</td>
</tr>
<tr>
<td>βV6(a)</td>
<td>0.0, 0.0, 0.0</td>
<td>0.2, 0.2, 1.3</td>
<td>0.1, 0.0, 0.3</td>
</tr>
<tr>
<td>αV2(a)</td>
<td>1.5, 3.5, 3.0</td>
<td>1.3, 7.3, 3.1</td>
<td>1.0, 1.1, 2.0</td>
</tr>
</tbody>
</table>

AGM PBL were stained with CD4, CD8 and respective anti-TCR antibodies and the distribution of T cell repertoires in each subset designated in Fig. 1(C) are shown.
Fig 4. CD4 expression in freshly isolated (I) and cultured CD4 cells (J–L) of AGM. FSC versus SSC dot blots of freshly isolated CD4CD8<sup>low</sup> cells (A) and cultured CD4 cells (B–D). The square in each figure indicates the scatter gate. (E–H) Negative control cells stained with control PE- and FITC-IgG antibodies. The profile of unsorted PBL is given in (E) as the negative control for isolated CD4CD8<sup>low</sup> cells (I). In (F), ~10% of blast cells were PE-positive because of the residual fluorescence of CD4 antibody employed for cell sorting. The residual fluorescence was also recognized when no control antibodies were used. (I) Sorted CD4CD8<sup>low</sup> cells. CD4<sup>+</sup> cells, 99.9%. (J) Cultured CD4 cells at 4 days. CD4<sup>+</sup> cells, 70%. (K) Cultured CD4 cells at 8 days. CD4<sup>+</sup> cells, 22%. (L) Cultured CD4 cells at 12 days. CD4<sup>+</sup> cells, 1%.

Fig. 5. Intracellular expression of CD4 antigen in cultured CD4 cells stained with CD4 (PE-Leu3a) antibody (upper row). Negative control cells were incubated with PE-IgG after staining of cell surface antigen (lower row). The histograms of red fluorescence are shown. No detectable CD4 antigen was recognized.

Fig. 6. Semiquantitative RT-PCR analysis of CD4, CD8α and CD8β gene expression in cultured CD4 cells. Peripheral CD8<sup>bright</sup> cells were also isolated and cultured for a positive control of CD8β gene expression. The total RNA was isolated from cultured cells at the points of cultivation as indicated. After cDNA synthesis, serial dilutions of cDNA in water were performed and the resultant solution was subjected to PCR assay. In cultured CD4 cells, almost no CD4 mRNA could be detected even in the undiluted cDNA sample at 12 days of cultivation.

which corresponded to the human CD4 molecule. It was inferred therefore that the CD4 molecule itself must disappear from the cell surface.

CD4 masking can be induced by the internalization of CD4 molecules (27,28); however, the accumulation of intracellular CD4 molecules was not recognized after activation. The results of RT-PCR indicated that the down modulation of cell surface CD4 molecules might be regulated in transcription level. Lymphoid cells migrate into the thymus and proliferate; they then rearrange their TCR genes and differentiate into mature T cells expressing either CD4 or CD8 antigens (29).

The exclusive expression of the CD8 antigen in most thymic CD4 cells may be incomplete in AGM and it is conceivable
Table 3. Helper activities associated with the IgG production of peripheral CD4CD8\textsuperscript{low}, CD8\textsuperscript{low} and CD8\textsuperscript{bright} cells

<table>
<thead>
<tr>
<th>Monkey</th>
<th>IgG ng/ml</th>
<th>B alone</th>
<th>B + 5% CD4CD8\textsuperscript{low}</th>
<th>B + 25% CD4CD8\textsuperscript{low}</th>
<th>B + 25% CD8\textsuperscript{low}</th>
<th>B + 50% CD8\textsuperscript{low}</th>
<th>B + 25% CD8\textsuperscript{bright}</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGM1</td>
<td>16</td>
<td>206</td>
<td>685</td>
<td>310</td>
<td>590</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>AGM2</td>
<td>26</td>
<td>58</td>
<td>399</td>
<td>207</td>
<td>356</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>AGM3</td>
<td>20</td>
<td>NT</td>
<td>NT</td>
<td>90</td>
<td>294</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

NT, not tested.

CD4CD8\textsuperscript{low}, CD8\textsuperscript{low} and CD8\textsuperscript{bright} cells were sorted out aseptically from PBMC. The numbers of cells of each fraction indicated in the table were added to 2×10\textsuperscript{5} autologous B cells. The mixtures were cultured in the medium containing PWM for 1 week. The IgG released in the culture supernatant was estimated by an indirect ELISA.

that cultured CD8 cells derived from peripheral CD8\textsuperscript{bright} lymphocytes expressed the CD8\textsuperscript{b} gene in addition to the CD8\textsuperscript{a} gene. Therefore, cultured CD4 cells and peripheral CD8\textsuperscript{bright} cells belong to a distinct T cell lineage in spite of similar antigen intensities of CD8\textsuperscript{a} molecules.

The finding of helper activity in the peripheral CD8\textsuperscript{low} population was interesting. This strongly suggested that the phenotypic conversion from CD4\textsuperscript{+} to CD4\textsuperscript{–} helper T cells occurred in vivo and that the CD4 masking was maintained while primed helper T cells developed into memory cells. The distribution of CD27 antigen also supports that peripheral CD8\textsuperscript{low} cells contain primed T cells which are generated by extensive cellular division. Accumulation of peripheral CD8 helper T cells might serve to prevent the emergence of a fatal immunodeficient state caused by SIV infection. The present data strongly indicate that CD4CD8\textsuperscript{low} cells mask the CD4 expression following lymphocyte activation which results in resistance to SIV. AGM may have acquired this unique differentiation of helper T cells as an adaptation to SIV. This defensive mechanism permits the co-existence of SIVagm and its host without an AIDS-like disease. We postulate that AGM have acquired a defensive mechanism, i.e. a CD4 masking of helper T cells, against SIV infection, so that they do not fall into a fatal immunodeficient state because CD4\textsuperscript{–} helper T cells escape SIV infection.

Recently, two chemokine receptors necessary for HIV-1 entry into CD4\textsuperscript{+} cells have been identified: CXCR-4 (fusin) is a co-receptor for entry of T cell line tropic virus (34) and CCR-5 is the second receptor for entry of macrophage tropic HIV strains (35–37). We cloned and sequenced these chemokine receptor genes in AGM, and the AGM CXCR-4 and CCR-5 genes are 97–98% identical to the human respective nucleotide sequences. We confirmed that CXCR-4 and CCR-5 mRNA could be detected in cultured, proliferating CD4 cells, in contrast to CD4 gene expression. Therefore, resistance to SIV infection in AGM observed in this study is not probably dependent on the expression of these co-receptor molecules, even if the same co-receptors are utilized by SIVagm.

AGM is expected to provide a useful model for studying the regulation of CD4 and CD8 expression in T cell differentiation. In the thymus, CD4 regulation in AGM appears to be similar to that in mice and human. After T cell maturation, IL-4 mediates CD8 induction on human CD4 cells (22) whereas such interleukin suppresses the CD8 expression on mouse CD8 cells (32). Although induction of CD8 in human CD8 cells by certain virus infections has been reported (33), detailed information on the regulation of CD4 gene expression by physiological stimuli is not yet available. The present data obtained in AGM CD4CD8\textsuperscript{low} suggest that unknown mechanisms may operate for the CD4 gene expression.

CD8\textsuperscript{a} antigen expression in cultured CD4 cells appeared to be somewhat increased as compared with those of small, resting CD4CD8\textsuperscript{low} cells. We suppose that this observation was probably due to the increased auto fluorescence of large, proliferating cells. CD8\textsuperscript{b} gene expression could not be detected in cultured CD4 cells as well as freshly isolated CD4CD8\textsuperscript{low} cells. These results were in contrast to the fact
fully activated proliferating cells. The efficiency of antigen recognition by TCR might be low after loss of the CD4 molecule in MHC class II-restricted cells. If the binding between MHC–antigen and the TCR is strong enough for induction of the following T cell responses, CD4 may not be essential for antigen recognition in AGM. It is possible that the CD4 masking phenomenon by the helper T cell itself could be applied to AIDS therapy if the regulatory factors estimated above are identified in the future.

**Abbreviations**

AGM  African green monkey  
AIDS  acquired immunodeficiency syndrome  
CCR-5  CC chemokine receptor 5  
Con A  concanavalin A  
CPE  cytotoxic effect  
CXCR-4  CXC chemokine receptor 4  
HIV  human immunodeficiency virus  
PBL  peripheral blood lymphocyte  
PBMC  peripheral blood mononuclear cell  
PE  phycoerythrin  
PerCP  peridinin chlorophyll protein  
PWM  pokeweed mitogen  
SEB  staphylococcal enterotoxin B  
SED  staphylococcal enterotoxin D  
SIV  simian immunodeficiency virus

**References**


CD4 and CD8 expression in monkey helper T lymphocytes


