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Phenotypic analysis of complement receptor 2^+ T lymphocytes: reduced expression on $CD4^+$ cells in HIV-infected persons

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SUMMARY

While expression of complement receptor 2 (CR2) (CD21) on some $CD4^+$ cell lines renders them more susceptible to infection by complement-treated human immunodeficiency virus (HIV), co-expression of CR2 and CD4 on peripheral blood lymphocytes has not, until recently, been observed. Several recent studies, however, have found that human T lymphocytes express low levels of CR2. Additionally, complement treatment of HIV before addition to these cells has been reported to increase virus expression in peripheral blood lymphocyte cultures. These findings suggest that complement-mediated enhancement of infection of human T cells could occur *in vivo* and have prompted us to examine both the phenotypic properties of $CD4^+CR2^+$ T cells in healthy persons and the expression of CR2 on $CD4^+$ lymphocytes during HIV infection.

As was previously reported, we observed CR2 on a proportion (10–50%) of both $CD8^+$ and $CD4^+$ T cells. Approximately half of $CD4^+CR2^+$ cells expressed the memory cell markers CD45RO and CD29, 80% expressed the naive marker CD45RA, while 22% expressed CD25. These values were not substantially different from total $CD4^+$ cells. Stimulation of lymphocytes with phytohaemagglutinin (PHA), OKT3 or calcium ionophore but not with phorbol myristate acetate (PMA) or interleukin-2 (IL-2) decreased expression of CR2 on CD4 cells by half over a 3-day culture period.

The per cent of $CD4^+$ cells expressing CR2 was significantly decreased in patients with asymptomatic and symptomatic HIV infection compared to uninfected control donors ($P=0{\cdot}0001$). In contrast, the decrease in CR2 expression was not observed with $CD8^+$ lymphocytes from HIV-infected persons. These results confirm that CR2 is expressed on human T lymphocytes and suggest that a subset of $CD4^+$ lymphocytes is selectively affected in HIV-infected individuals.

INTRODUCTION

Complement receptors (CR) for C3 are present on many cell types and are known to play roles in phagocytosis, clearance of immune complexes, and modulation of cell function.[1,2] In general, T lymphocytes have not been recognized as expressing CR. However, recent studies have described low level expression of CR1[3,4] and CR2[5] on subsets of human T cells and CR2 on human thymocytes.[6] The possible presence of CR2 on helper T cells is of particular interest since this CR has been shown to be involved in complement-mediated enhancement of human immunodeficiency virus (HIV) infection on some $CR2^+CD4^+$ cell lines.[7–10]

CR2 is a 140,000 Da glycoprotein which has binding specificity for the C3 cleavage fragments C3dg, C3d, and iC3b.[11–13] CR2 has also been identified as the receptor for Epstein-Barr virus (EBV)[13,14] infection since it binds to the viral envelope protein gp350/220 and is expressed on B lymphocytes.

Cross-linking of CR2 has been shown to synergize with an anti-IgM stimulus in increasing intracytoplasmic Ca^{2+} in B lymphocytes.[15] CR2 could also be involved in T-cell signal transduction or aid in T-cell adhesion to complement-coated surfaces. Since only a proportion of T cells express CR2,[5] these cells may represent a different stage of maturation/differentiation than $CD4^+CR2^-$ cells. This study was undertaken to confirm expression of CR2 on T cells and describe the phenotype of the $CD4^+$ cells which express this CR. The expression of CR2 on $CD4^+$ cells from HIV-infected persons was also studied, since cell lines which express both CD4 and CR2 have been shown to exhibit complement mediated enhancement of HIV infection.

MATERIALS AND METHODS

Cell isolation

Peripheral blood mononuclear cells were isolated from heparinized blood by centrifugation on Ficoll-Hypaque.[16] Samples

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were obtained from healthy donors (ages 22–49) and persons diagnosed with various stages of HIV infection (asymptomatic, symptomatic non-AIDS, or AIDS) based on the Centers for Disease Control criteria.[17,18] Informed consent was obtained from all donors. The mononuclear cell layer was washed 2× and resuspended in RPMI-1640 medium (Gibco, Grand Island, NY). Red blood cells (RBCs) were obtained from the bottom RBC pellet of the Ficoll-Hypaque gradients. The RBCs were washed 2× (removing the buffy layer each time) and resuspended in RPMI-1640.

Monoclonal antibodies

Murine monoclonal antibodies (mAb), either unconjugated or directly conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or biotin, were used to stain isolated mononuclear cells and red blood cells. Monoclonal Ab recognizing three different epitopes of CD21 (CR2) were used including unconjugated and FITC-labelled BL13[19] (a generous gift from Connie Etheridge, AMAC, Westbrook, ME), unconjugated HB-5[20] (Becton Dickinson, Mountain View, CA), and unconjugated OKB7[21] (Ortho Diagnostics, Raritan, NJ). Other mAb also used in two- and three-colour staining experiments included anti-CD4 (PE and Biotin) (a generous gift from Connie Etheridge, AMAC), CD8 (Leu 2a-PE), CD3 (Leu 4-PE), CD14 (Leu M3-FITC), CD19 (Leu 12-FITC), CD11b (Leu 15-FITC), CD25 (IL-2R-PE), CD35 (CR1-unconjugated), HLA-DR-FITC (Becton Dickinson), CD29 (4B4-PE), CD45RA (2H4-PE) (a kind gift from Robert Raynor, Coulter, Hialeah, FL), and CD45RO (UCHL1-PE) (DAKO, Carpinteria, CA). FITC-labelled goat anti-mouse-Ig (FITC-GAM) (TAGO, Burlingame, CA) and streptavidin DuoChrome (Becton Dickinson) were used as sources of secondary staining reagents.

Cell staining and flow cytometric analysis

CR1 levels on erythrocytes were determined by a modification of the method of Cohen *et al.*[22] For one-colour staining of RBCs, cells were incubated with unconjugated anti-CR1 mAb for 30 min on ice, washed, and then incubated with FITC-GAM. The mean linear fluorescence was determined by subtraction of background staining, using an isotype-matched control mAb, from anti-CR1 staining.

For two-colour staining of lymphocytes, cells were incubated with unconjugated anti-CR2 mAb for 30 min on ice, washed, and then incubated with FITC-GAM for an additional 30 min on ice. Stained cells were incubated with 1% mouse serum for 20 min on ice prior to addition of a second PE-conjugated mouse mAb for 30 min on ice.

For three-colour staining of lymphocytes, cells were incubated with all three primary mouse mAb (FITC, PE, and Biotin conjugated) for 30 min on ice, washed, and then incubated with Streptavidin DuoChrome for an additional 30 min on ice.

Samples were fixed with 1% paraformaldehyde and analysed on a FACScan flow cytometer (Becton Dickinson). The data were collected using Consort 30 (two colour) or FACScan Research software (three colour), from 10,000–50,000 gated (forward scatter versus 90° light scatter) events and expressed as log fluorescence intensity. Samples were analysed using LYSYS software (Becton Dickinson).

For both two- and three-colour analysis of lymphocytes, background fluorescence was established at <3% using an isotype-matched control mAb and subtracted from the percentage of positive cells.

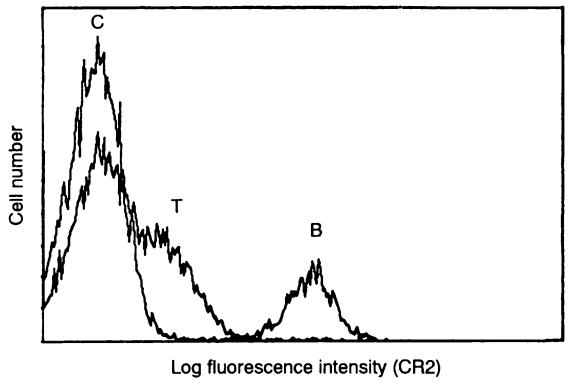

Figure 1. Comparison of CR2 levels on peripheral blood B and T lymphocytes. Peripheral blood mononuclear cells were stained with purified anti-CD21 (BL13) or an isotype-matched control antibody. An FITC-labelled secondary staining reagent was used for detection. Cells were co-stained with either PE-labelled anti-CD3 (T cell) or anti-CD19 (B cell) mAb. The expression of CR2 on B lymphocytes (B) and on T lymphocytes (T) is shown. Background staining with the isotype-matched control antibody on T cells is shown (C).

Lymphocyte stimulation

Isolated peripheral blood mononuclear cells were cultured in complete media (CM) consisting of RPMI-1640 supplemented with 10% heat inactivated foetal calf serum (FCS) (HyClone, Logan, UT) and 50 μg/ml gentamicin (Sigma, St Louis, MO). Cells were stimulated with either 3 μg/ml PHA-P (Sigma) plus 5% IL-2 supernatant (Cellular Products, Buffalo, NY) in CM; 5% IL-2 supernatant only in CM; PMA (Sigma) (0·5 μg/ml) in CM; calcium ionophore A23187 (Calbiochem, San Diego, CA) (2×10^{-5} M) in CM; or OKT3 (Ortho Diagnostics) (100 ng/ml) (using a modification of the method of van Wauwe[42]) in RPMI-1640 supplemented with 50% v/v heat inactivated AB^+ human serum. Cultures were stimulated for 3 days at a concentration of 2×10^6 cells/ml, washed, and stained as indicated above for two-colour staining.

The following formula was used to calculate the per cent decrease in CR2 expression on cultured $CD4^+$ cells:

Per cent decrease = [1 − (per cent of $CD4^+$ cells which express CR2 subsequent to stimulation/per cent of $CD4^+$ cells which express CR2 prior to stimulation)] × 100.

RESULTS

Expression of CR2 (CD21) on T lymphocytes

A previous study reported low level expression of CR2 on T lymphocytes.[5] To confirm the results of that study, mononuclear cells were isolated from healthy donors and the levels of CR2 (BL13 epitope) were assessed on both T cells and B cells by two-colour flow cytometry (Fig. 1). B lymphocytes ($CD19^+$) expressed high levels of cell surface CR2 (fluorescence intensity = 131), while T cells ($CD3^+$) expressed low levels of cell surface CR2 (fluorescence intensity = 39). The fluorescence intensity of background staining with an isotype-matched control mAb was 26. The fraction of T lymphocytes ($CD3^+$),

Table 1. Expression of CR2 on T lymphocyte populations

Sex	CD3[a]	CD4	CD8
Males	30±8 [20–38] (n=4)[b]	33±9 [12–49] (n=14)	29±9 [16–38] (n=8)
Females	ND[c]	30±6 [26–41] (n=10)	24±6 [17–28] (n=5)
Ave.	—	32±7 [12–49] (n=23)	27±8 [16–38] (n=13)

[a] Cells in the lymphocyte gate (gated by forward versus 90° light scatter) were assessed for the per cent of CD3$^+$, CD4$^+$, and CD8$^+$ cells which co-expressed the CR2 (CD21) epitope recognized by BL13. For all samples, background fluorescence was established (< 3%) using an isotype-matched control and subtracted from the per cent positive cells.

[b] Values represent mean per cent ± standard deviation. The range is shown in brackets and the number of persons tested is shown in parentheses.

[c] ND—not determined.

The per cent of CD4$^+$ and CD8$^+$ cells expressing CR2 were not significantly different (t-test, $P > 0.05$). Males and females were not significantly different in the expression of CR2 on either CD4$^+$ or CD8$^+$ cells (t-test, $P > 0.05$).

This table represents a compilation of data on control donors from Figs 2 and 3 as well as other data not shown elsewhere.

Table 2. Expression of CD21 epitopes on CD4$^+$ cells

Subject	HB5[a]	BL13	OKB7	CD19
1	27	ND[b]	30	0
2	45	46	50	1
3	7	12	10	0

[a] Cells in the lymphocyte gate (gated by forward versus 90° light scatter) were assessed for the per cent of CD4$^+$ cells which co-express either one of three CR2 (CD21) epitopes (HB5, BL13, or OKB7) or a B-lymphocyte marker (CD19).

[b] Not determined.

For all samples, background fluorescence was established (< 3%) using an isotype-matched control and subtracted from the per cent positive cells.

Table 3. Reduction in CR2 induced by stimulation of CD4$^+$ T lymphocytes

Culture conditions[a]	% reduction ± SEM (# exps)[b]
IL2 only	3±13 (5)
PHA+IL2	55±8 (6)
OKT3	55±12 (3)
Ionophore	46±17 (3)
PMA	−2±6 (3)

[a] Cells were cultured in CM supplemented with either 5% IL2 only, CM supplemented with 5% IL2 and PHA (3 μg/ml), OKT3 (100 ng/ml), Ionophore (2×10^{-5} M), or PMA (0·5 μg/ml).

[b] Values represent mean per cent decrease (± standard error of the mean) of CD4$^+$CR2$^+$ cells compared to freshly isolated (time 0) cells as determined by the following formula: per cent decrease = [1 − (per cent of CD4$^+$ cells which express CR2 subsequent to stimulation/ per cent of CD4$^+$ cells which express CR2 prior to stimulation)] × 100. The number of experiments is shown in parentheses. Negative value (PMA treatment) represents an increase in per cent of CR2-CD4 positive cells.

helper T lymphocytes (CD4$^+$), and suppressor T lymphocytes (CD8$^+$) which co-expressed CR2 was also determined by two-colour flow cytometry (Table 1). An average of 30% of T lymphocytes expressed CR2, while the proportions of CD4$^+$ and CD8$^+$ cells which were CR2$^+$ (32% and 27% respectively) were not significantly different (t-test, $P > 0.05$).

To exclude the possibility that the anti-CR2 mAb BL13, which was used in the above experiments, was cross-reacting with non-CR2 determinants on CD4$^+$ T lymphocytes, two other mAbs (HB5 and OKB7) directed against different epitopes of CR2[23] were used to analyse CR2 expression by CD4$^+$ cells (Table 2). All three monoclonal antibodies co-stained CD4$^+$ T cells with similar percentages indicating the presence of CR2. In contrast, CD19, which is a B-lymphocyte lineage-specific marker, was not detected on helper T cells. The lack of co-expression of CD19 with CD4 excludes the possibility that B cells in some way accounted for the CR2 expression on CD4$^+$ cells.

To determine if there was a differential expression of CR2 on CD4$^+$ or CD8$^+$ T lymphocytes associated with gender, CR2 was measured in both males and females. The results indicated that there is a wide range of expression of CR2 in the normal population, but males and females did not express significantly different levels (t-test, $P > 0.05$) (Table 1).

Since expression of many cell surface receptors changes after stimulation,[24] the expression of CR2 on helper T lymphocytes was examined after 3 days of culture in the presence of various cell stimulators (Table 3). A substantial reduction in CR2

Table 4. Phenotypic analysis of the activation/differentiation state of $CD4^+CR2^+$ T lymphocytes

Cell surface marker	Total CD^{+a}	$CD4^+CR2^+$
CD45RA	78 ± 17^b	82 ± 13
CD45RO	49 ± 11	45 ± 14
CD29	44 ± 11	41 ± 20
CD25	32 ± 5	22 ± 8

[a] Cells in the lymphocyte gate (gated by forward versus 90° light scatter) were assessed for the percentage of $CD4^+$ and $CD4^+CR2^+$ cells which co-expressed the epitopes CD45RA, CD45RO, CD29, and CD25 (IL-2R). For all samples, background fluorescence was established at <3% using an isotype-matched control and subtracted from the per cent positive cells.

[b] Values represent mean per cent ± SD of four persons.

expression by $CD4^+$ cells was observed after stimulation with PHA (55% reduction in CR2), OKT3 (55% reduction in CR2), or calcium ionophore (46% reduction in CR2). In contrast, little or no reduction in CR2 expression was observed after stimulation with PMA or IL-2 only. Incubation in medium only also had no effect on CR2 expression on $CD4^+$ cells (data not shown).

Phenotypic analysis of the activation/differentiation state of $CD4^+CR2^+$ T lymphocytes

Previous studies have indicated that $CD4^+$ cells can be characterized as 'naive' or 'memory' cells depending on expression of certain cell surface markers. Three-colour flow cytometry was used to characterize the maturation state of the $CR2^+$ helper T cells. Both total $CD4^+$ and the $CD4^+CR2^+$ subset expressed similar percentages of the cell surface markers CD45RA, CD45RO, CD29, and IL-2R (Table 4). A majority of the cells expressed the naive cell marker CD45RA, but approximately half of the cells expressed the memory cell markers CD45RO and CD29. Thus, some $CD4^+CR2^+$ cells appear to express both naive and memory markers. Co-expression of naive and memory markers on a proportion of peripheral blood lymphocytes has previously been described.[25,26]

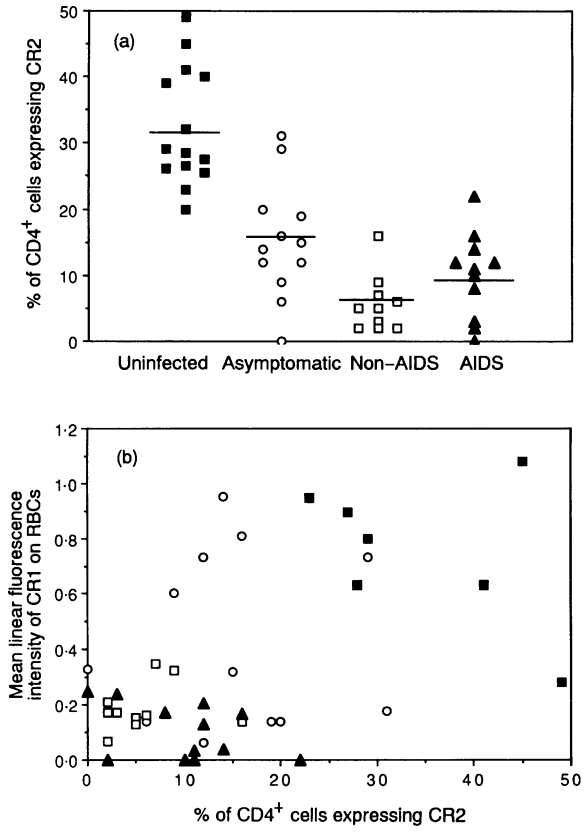

Figure 2. Analysis of CR2 expression on $CD4^+$ lymphocytes and CR1 levels on erythrocytes from HIV-infected and uninfected persons. (a) Peripheral blood mononuclear cells were stained with purified anti-CD21 (BL13) and FITC-GAM. Cells were co-stained with PE-labelled anti-CD4 mAb. The percentage of $CD4^+$ cells expressing CR2 is shown for samples obtained from HIV-uninfected and HIV-infected donors. Line represents mean of each group. (b) For some of the donors shown in Fig. 2a, CR1 levels on erythrocytes were also determined using a purified anti-CR1 mAb. The percentage of $CD4^+$ cells expressing CR2 is displayed on the x axis, while CR1 expression on erythrocytes is shown on the y axis (see Methods). A correlation coefficient was determined using all patient and control donor data: $r = 0{\cdot}506$, $P = 0{\cdot}007$ (analysis of variance). In Fig. 2a and b, samples were obtained from the following donors: HIV-uninfected, closed squares; HIV-infected asymptomatic, open circles; symptomatic non-AIDS, open squares; AIDS, closed triangles.

Analysis of $CD4^+CR2^+$ T lymphocytes from HIV-infected persons

Since $CD4^+CR2^+$ cell lines are more efficiently infected by complement-treated virus, we investigated the possibility that the $CD4^+CR2^+$ subset was affected in HIV-infected persons. As a positive control for changes in HIV-infected persons, CR1 levels on erythrocytes were evaluated since previous studies have shown that during progression of HIV disease, CR1 levels decrease on erythrocytes.[27-29]

While $32 \pm 9\%$ ($n = 14$) of $CD4^+$ cells were $CR2^+$ in uninfected control donors, significant ($P = 0{\cdot}0001$, analysis of variance) decreases in CR2 expression on $CD4^+$ cells were found at all stages of HIV infection (Fig. 2a). The mean per cent ± SD of $CD4^+$ cells expressing CR2 in HIV-infected donors were as follows: asymptomatic, $16 \pm 9\%$ ($n = 12$); symptomatic non-acquired immune deficiency syndrome (AIDS), $6 \pm 4\%$ ($n = 10$); and AIDS, $10 \pm 6\%$ ($n = 11$) (Fig. 2a). As has been reported previously[27,28] the level of CR1 on erythrocytes from HIV-infected persons was significantly decreased ($P = 0{\cdot}0001$, analysis of variance) when compared to HIV-uninfected control donors (Fig. 2b). A significant correlation ($r = 0{\cdot}506$, $P = 0{\cdot}007$) was observed between the levels of $CR2^+CD4^+$ T lymphocytes and the levels of CR1 on erythrocytes.

The activation/differentiation phenotype of $CD4^+CR2^+$ T lymphocytes from HIV-infected donors [asymptomatic ($n = 3$), symptomatic non-AIDS ($n = 3$), and AIDS ($n = 4$)] was also examined. There were no notable differences, comparing all groups of HIV-infected to uninfected control donors, found in the percentages of $CD4^+CR2^+$ cells expressing CD45RA, CD45RO, CD29, or IL-2R (data not shown).

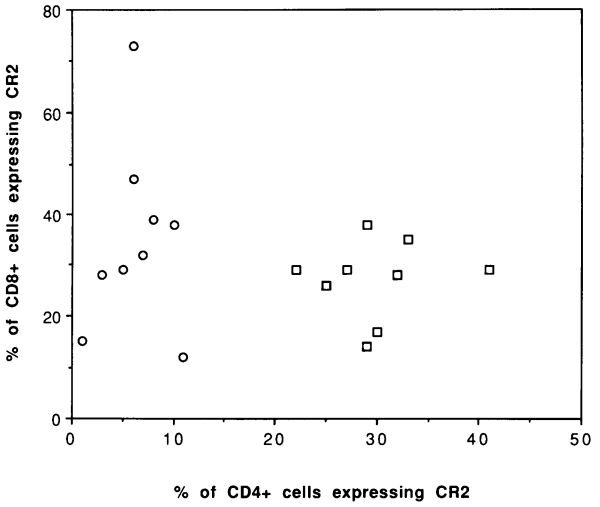

Figure 3. Comparison of CR2 expression on $CD4^+$ and $CD8^+$ T lymphocytes from HIV-infected and uninfected persons. Peripheral blood mononuclear cells were stained with purified anti-CD21 (BL13) and FITC-GAM. Cells were co-stained with either PE-labelled anti-CD4 or anti-CD8 mAb. The percentage of $CD4^+$ cells (x axis) and $CD8^+$ cells (y axis) expressing CR2 is shown. Samples were obtained from nine HIV-uninfected (open squares) and nine HIV-infected (open circles) donors (two AIDS and seven symptomatic non-AIDS).

To determine if the decreased CR2 expression in HIV-infected persons was specific for the $CD4^+$ subset of lymphocytes, the percentage of both $CD4^+$ and $CD8^+$ cells which expressed CR2 were determined for 13 additional HIV-infected and nine additional HIV-uninfected controls (Fig. 3). As was found in the first group of HIV-infected persons and controls (Fig. 2a), a significantly lower proportion (*t*-test, $P<0{\cdot}002$) of $CD4^+$ lymphocytes were $CR2^+$ in HIV-infected persons ($6\pm3\%$ $n=9$ [two AIDS and seven symptomatic non-AIDS] [four of the 13 patients had too few $CD4^+$ cells to assess]) when compared to control donors ($30\pm5\%$) (Fig. 3). In contrast, no significant decrease (*t*-test, $P>0{\cdot}05$) in $CD8^+CR2^+$ numbers was observed in HIV-infected persons when compared with controls ($32\pm16\%$, $n=13$ and $27\pm8\%$, $n=9$ respectively).

One possible explanation for the decrease in CR2 expression on $CD4^+$ cells but not on $CD8^+$ cells in HIV-infected persons could be that direct infection of the $CD4^+$ cells *in vivo* caused loss of expression of CR2. To evaluate the effect of HIV infection on CR2 expression, MT2 cells, which express both CD4 and CR2 were infected with HIV. After 4 days of culture, both the percentage of $CR2^+$ cells (94%) and the relative level of CR2 expression were the same for infected and uninfected MT2 cells (data not shown). In contrast, expression of CD4 was reduced from 79% $CD4^+$ in uninfected cultures to undetectable levels (0% positive) in infected cultures as has been previously described[30] for *in vitro* infected cells.

DISCUSSION

Recently, Fischer *et al.*[5] reported low level expression of CR2 on human peripheral blood T lymphocytes. The current study confirms those results by showing that expression of CR2 occurs at similar levels on subsets of both $CD4^+$ and $CD8^+$ T lymphocytes.

It was also observed in this study that $CD4^+CR2^+$ cells are predominantly $CD45RA^+$. However, slightly less than half the $CD4^+CR2^+$ cells also express CD45RO and CD29. Helper T cells can be divided into naive and memory subpopulations based on expression of the surface markers CD45RA and CD45RO respectively.[25,31] Helper T cells which express increased levels of CD29 are also identified as memory cells.[25,31] Thus, although the $CD4^+CR2^+$ cells found in this study belong predominantly to the 'naive' subset of helper T cells, a substantial proportion of these cells also express 'memory' markers. We therefore conclude that both memory and naive T cells can express CR2. However, PHA stimulation, which decreases CD45RA while increasing CD45RO and CD29 expression, caused a decrease in CR2 expression, suggesting that CR2 expression may be more closely linked to naive cells.

At least one previous study could not detect CR2 on peripheral blood T lymphocytes or thymocytes.[20] However, Tsoukas *et al.*[6] have shown that CR2 is expressed at low levels on 15–63% of thymocytes. Since the expression of CR2 is low on both T cells and thymocytes, it is possible that the expression was below the level of detection in the earlier study.

We also observed a significant decrease in the percentage of $CD4^+$ T cells expressing CR2 during HIV infection. It is unlikely that opportunistic infections are causing the decreased CR2 expression, since significant decreases were found in HIV-infected asymptomatic persons. There are several other possible explanations for this decrease. First, it has been shown that immune complexes are present in HIV-infected persons.[32,33] It is possible that complement breakdown products C3dg and C3d on immune complexes could cross-link CR2 on T cells, thus stimulating them and decreasing CR2 expression. It is unlikely, however, that the decreased CR2 expression found in this study was due to immune complex blocking of the BL13 mAb epitope, since BL13 and C3d bind to separate sites on CR2.[19,34]

In this study we observed that *in vitro* activation of cells by PHA, calcium ionophore, or OKT3 caused a decrease in CR2 expression on T cells. Thus, *in vivo* activation of these cells during HIV infection could also explain the decreased expression.

HIV infection could result in reduction in the $CD4^+CR2^+$ cell population by at least two mechanisms. First, HIV has been shown to decrease expression of cell surface markers[30,35] in infected cells. However, it is unlikely that HIV decreases CR2 expression since we observed that in HIV-infected MT2 cells CD4 was decreased, but CR2 was not affected. Also, only 1/100 to 1/1000 $CD4^+$ peripheral blood lymphocytes contain HIV provirus.[36,37] Viral infection of cells is thus too infrequent to explain the observed CR2 decrease. However, a second possible mechanism is that preferential infection of $CD4^+CR2^+$ cells could result in their subsequent destruction and depletion, which could explain the decreased number of $CD4^+CR2^+$ cells found in infected persons. In support of this, several groups[10,38] have reported that HIV infection of cell lines which express both CD4 and CR2 is enhanced after antibody and complement treatment of virus. This enhanced infection appears to result from increased binding and entry of HIV into $CD4^+CR2^+$ cells[7] due to binding of cell surface CR2 to complement breakdown products on the surface of the virus.[10] *In vivo*, $CD4^+CR2^+$ cells could either be killed directly due to infection or indirectly by

anti-viral immune mechanisms. This hypothesis is supported by the observation that in HIV infection, the $CD4^+CR2^+$ cells but not the $CD8^+CR2^+$ cells are relatively decreased.

To date, complement-mediated enhancement of HIV infection of cells has been shown mainly to occur in cell lines.[8-10,38,39] However, two studies have also found that complement-enhanced infection can occur in peripheral blood lymphocytes.[40,41] We have shown that there is a subpopulation of helper T lymphocytes *in vivo* which express the receptors required for complement-mediated enhancement of HIV infection. We have also shown that this subpopulation is decreased in HIV-infected persons. Further *in vitro* studies are currently being performed to determine whether $CD4^+CR2^+$ cells are infected preferentially by antibody-plus-complement-treated HIV.

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