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Relationship of antibodies against CD4$^+$ T cells in HIV-infected patients to markers of activation and progression: autoantibodies are closely associated with CD4 cell depletion

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SUMMARY

Antibodies against lymphocytes have been shown in human immunodeficiency virus (HIV)-infected patients, but their relevance in the pathogenesis of acquired immune deficiency syndrome (AIDS) remains controversial. We investigated increased levels of lymphocyte surface Ig and antibodies against CD4$^+$ T cells in the plasma. The relationship to CD4 cell depletion and serological parameters were analysed. A three-colour flow cytometric method was used to detect surface Ig on the surface of patients' cells and antibodies in the plasma of the patients. We observed a high percentage of patients with increased surface Ig on CD4$^+$ T cells (94%—47/50). Antibodies in the plasma reacting with healthy donors' CD4$^+$ T cells were detectable in 72% (23/32) of the patients. CD4 cell-surface Ig correlated well with surface Ig on different T-cell subpopulations but not with increased surface Ig on B cells. Only one control showed elevated surface Ig, plasma antibodies against lymphocytes were not detectable. Surface Ig levels of CD4$^+$ T cells were closely associated with the CD4 cell number in HIV-infected patients of all stages of disease ($r = -0{\cdot}67$, $P = 0{\cdot}00005$). Other lymphocyte subsets' surface Ig did not show a significant association to CD4 cell depletion. Surface Ig and antibodies against CD4$^+$ T cells were not related to levels of β_2-microglobulin, p24 antibodies or interleukin-6 (IL-6), and did not depend on hypergammaglobulinaemia. In conclusion surface Ig on CD4$^+$ T cells is likely to have an autoantibody origin. The high prevalence and association to CD4 depletion support the view that autoimmune phenomena could be involved in the pathogenesis of AIDS.

INTRODUCTION

The period of latency before acquired immune deficiency syndrome (AIDS) averages about 10 years after human immunodeficiency virus (HIV) infection.[1] The technical term 'latency period' seems to be misleading because CD4$^+$ T cells decrease continually in this period. AIDS, finally, develops in patients with less than 200 CD4$^+$ T cells/μl in peripheral blood.[2] The mechanisms which lead to the loss of helper cells during this period are not clearly understood,[3] although the number of cells infected with HIV may be higher than previously expected.[4] Other mechanisms than the cytopathic effect of the virus alone are supposed to contribute to the destruction of the immune system in HIV-infected patients. Immunological models have been published in the last few years suggesting a role of autoimmunity in the pathogenesis.[5,6] In fact there are many direct and indirect signs of autoimmune reactivity in HIV-infected patients.[7] The parameters of disease progression show a close association between activation of the immune system and development of AIDS, especially elevated serum levels of tumour necrosis factor-α (TNF-α) and interferon-α (IFN-α) indicate a poorer prognosis.[8,9]

T cells with increased levels of surface bound Ig have been shown in HIV-infected patients. These lymphocyte surface bound Ig are supposed to be, at least in part, autoantibody in nature.[10-13] The same authors and others[14,15] demonstrated anti-lymphocyte antibodies in the serum of patients with HIV infection by cytotoxic and fluorescence methods. Autoantibodies against the soluble form of the CD4 molecule[16,17] and other lymphocyte antigens[18,19] have been shown. Although autoreactivity and its relevance to the induction of immune dysbalance is widely discussed, there is no certainty about the role of these phenomena in the pathogenesis of AIDS.

The aim of this study was to investigate the proportion of HIV-infected patients with increased surface Ig on different lymphocyte subsets and the association to anti-lymphocyte antibodies in the plasma. The relationship of increased surface

Abbreviations: AIDS, acquired immune deficiency syndrome; ARC, AIDS-related complex; HIV, human immunodeficiency virus; Ig, immunoglobulin; IL-6, interleukin-6; LAS, lymphadenopathy syndrome.

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Ig to parameters of cellular activation and disease progression (especially CD4 cell depletion) was determined as well.

MATERIALS AND METHODS

Patients

The amount of surface Ig on $CD4^+$ T cells was measured in 50 HIV-1-infected patients. Antibodies in the plasma and different immune parameters were examined in 32 of the 50 HIV-1-infected patients. The patients were in different stages of disease: seven patients were asymptomatic, three presented with lymphadenopathy syndrome (LAS), 16 with AIDS-related complex (ARC) and 24 patients fulfilled the criteria for AIDS. The wide range of CD4 cell counts (8–878/μl) indicated the different degrees of immune destruction as well. Twenty healthy blood donors served as controls. Sera of 13 patients with systemic parasitic diseases [leishmaniasis (5), trypanosomiasis (2) and Chagas disease (6)] were used to check the influence of hypergammaglobulinaemia on the presence and distribution of immunoglobulins on lymphocytes.

Lymphocyte subpopulations

Lymphocyte subpopulations were determined in whole blood using double staining with monoclonal antibodies and a commercially available lysing solution (Becton Dickinson, Sunnyvale, CA). Leucocytes were quantified by standard methods and the absolute count of lymphocyte subpopulations was calculated.

Detection of surface bound immunoglobulin

Forty microlitres whole blood was washed twice with phosphate-buffered saline (PBS) at 160 *g* for 10 min to remove platelets which could absorb the anti-Ig antibody.

Twenty-five microlitres of diluted (1:4) goat $[F(ab')_2]$ polyclonal anti-human Ig GAM (Medac, Hamburg, Germany) was used to detect immunoglobulins on the cell surface. The incubation was for 30 min at 4°, followed by a wash cycle. Subsequently the samples were double stained with monoclonal antibodies conjugated with phycoerythrin (PE) and peridinin chlorophyll protein (PerCP) (all Becton Dickinson) as follows: Leu-3a (CD4), Leu-4 (CD3), Leu-2a (CD8), Leu-16 (CD20), HLA-DR.

Lysing solution was added for 10 min, the samples were washed three times and examined by a FACScan (Becton Dickinson). Depending on the CD4 cell count 5000 or 7500 lymphocytes were analysed (see Fig. 1).

The increase of surface bound Ig on different lymphocyte subsets was analysed regarding the percentage and the mean fluorescence intensity of increased surface Ig carrying cells. The mean fluorescence intensity was used to compare the amount of surface bound Ig in different patients and cell populations. In samples with less than 10% Ig^+ cells the mean fluorescence intensity of all cells was used for calculations.

Detection of anti-lymphocyte antibodies in the plasma

Anti-lymphocyte antibodies were investigated using a modified procedure described by Dorsett *et al.*[20]

Fifty microlitres of whole blood from a healthy donor (blood group O) was incubated with 135 μl of (control or patient) plasma for 45 min. To control hypergammaglobulinaemia we used a commercially available Ig preparation (Behring, Marburg, Germany) containing high amounts of Ig (160 mg/ml) in a physiological composition. Whole blood was incubated with 10 μl and 50 μl of this preparation as well. Cells were washed twice at 160 *g*. Subsequently, the cells were stained with anti-human Ig GAM and the monoclonal antibodies as described before.

Serological parameters

Serum IgG and IgM levels were measured by radial immunodiffusion (Behring). The measurement of interleukin-6 (R&D System, Minneapolis) and β_2-microglobulin (Behring) was done by commercially available ELISA test kits.

Antibodies against viral p24 in the patients' plasma were measured by Western blot analysis (BioRad, München, Germany) followed by semi-quantitative densitometry.

Statistical analysis

Linear correlations were calculated and proved significant with a two-tailed Student's *t*-test. Percentages and mean fluorescence intensities of (Ig^+) lymphocyte subpopulations were compared by Wilcoxon's signed rank test for paired groups. $P < 0.05$ was considered to be significant.

RESULTS

Lymphocyte subpopulations

Patients with HIV infection showed a significantly lower CD4 cell number in the peripheral blood than healthy controls. $CD3^+$ T cells were decreased, whereas no significant difference was seen when the B-cell counts of patients and controls were compared. Patients with HIV infection showed higher numbers of activated ($HLA\text{-}DR^+$) and $CD8^+$ T cells in peripheral blood than controls (see Table 1).

Surface bound immunoglobulin

Circulating T cells of healthy persons show very low levels of surface bound Ig. Surface Ig is increased on $HLA\text{-}DR^+$ T cells (due to the expression of Fc receptors) and B cells which constitutively carry Ig. Therefore the cut-off between normal and increased surface Ig had to be set differentially regarding different lymphocyte subsets.

Ninety-four per cent of the HIV-infected patients displayed an increased level of surface bound Ig on their T cells compared to healthy controls (Table 2). The majority of patients did not show an increase of the B-cell surface Ig, either by percentage or mean fluorescence intensity. Out of the controls (20) only one had elevated surface bound Ig. Sixty-three per cent of the T cells of this healthy person showed an increased amount of Ig, whereas in HIV-infected patients usually more than 90% of the T cells had elevated levels of surface bound Ig (see Fig. 2).

Different T-lymphocyte subpopulations in HIV infected patients did not reveal an obvious difference concerning the percentage of Ig^+ cells. But monitoring the mean fluorescence intensity of Ig^+ cells revealed that $CD4^+$ T cells had more surface bound Ig than other T-cell subpopulations (Fig. 3). The difference in the amount of surface Ig on $CD4^+$ and $CD8^+$ T cells was highly significant ($P < 0.0001$).

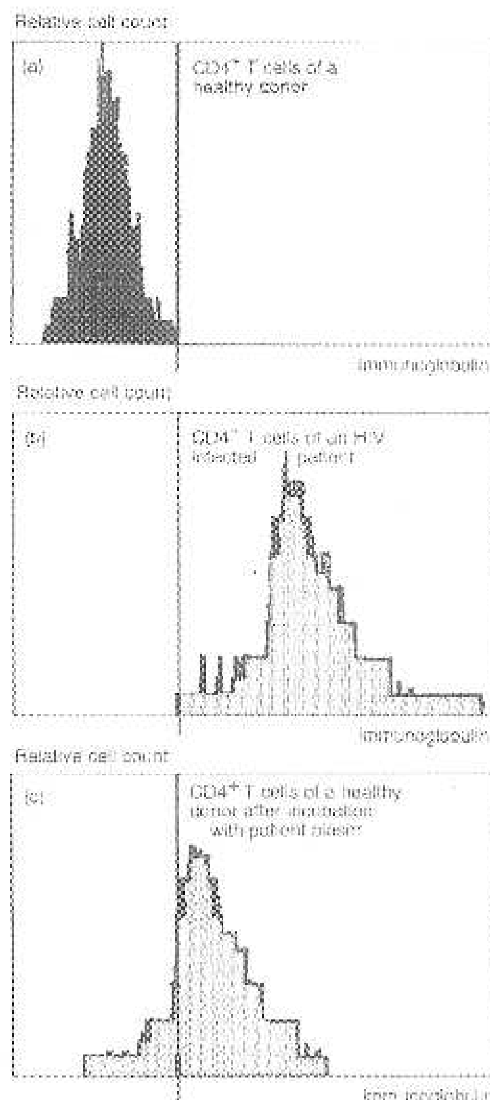

Figure 1. Measurement of surface Ig and antibodies against CD4+ lymphocytes: (a) surface Ig of a healthy donor; (b) detection of increased surface Ig and (c) detection of plasma antibodies against CD4+ lymphocytes. A patient was considered to be positive for increased surface Ig if at least 50% of his CD4+ T cells had abnormal high surface Ig. Plasma with Ig binding to > 10% of healthy donors' cells (CD4+) were considered to be positive for antibodies against CD4+ T cells. As well as the percentage of Ig+ cells, the MFI of Ig+ cells was measured.

Table 1. HIV+ patients show lower CD4 cell counts but an increase in activated (HLA-DR+) T cells. No significant difference concerning the number of B cells was observed

	HIV+ ($n=32$) (cells/μl)	Controls ($n=12$) (cells/μl)	Significance
Lymphocytes	1181 ± 585*	1388 ± 395	$P=0{\cdot}27^{NS}$
T cells	874 ± 269	1048 ± 176	$P=0{\cdot}0444$
Activated T cells	424 ± 219	175 ± 82	$P=0{\cdot}0004$
CD4+ T cells	213 ± 223	675 ± 172	$P<0{\cdot}0001$
CD8+ T cells	618 ± 278	358 ± 134	$P=0{\cdot}0036$
B cells	199 ± 128	135 ± 59	$P=0{\cdot}10^{NS}$

* Mean ± SD.

Table 2. The portion (number and percentage) of patients with increased surface Ig. Also average percentage of Ig+ cells [mean ± SD (%)] and range are shown (all patients included). The majority of patients show increased surface Ig on T cells, only a minor proportion elevated Ig on B cells. Within the 20 controls elevated surface Ig could be demonstrated in one person (1/20—5%) on 60% of the CD4+ T cells only

	n	%	Mean ± SD (%)	Range
T cells	30/32	94	94 ± 16	36–100
Activated T cells	30/32	94	91 ± 18	27–100
CD4+ T cells	47/50	94	95 ± 17	33–100
CD8+ T cells	29/30	97	94 ± 15	26–100
B cells	9/32	28	38 ± 21	2–77

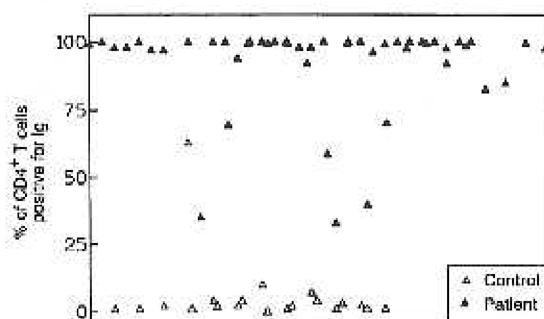

Figure 2. Surface bound Ig on CD4+ T cells. Increased surface Ig on CD4+ T cells was observed in almost all patients infected with HIV (94%, 47/50) as detected by the direct method. Only one control had elevated (> 50%) levels of bound Ig. Usually controls have < 10% Ig+ and patients > 90% Ig+ cells.

Antibodies in the plasma

To examine the origin of abnormal high surface Ig in HIV-infected patients we measured anti-CD4 cell antibodies in the plasma of the patients. Seventy-two per cent of the patients were found to be positive for plasma antibodies against CD4+ T cells. No antibodies against lymphocytes could be demonstrated in control plasma. Patients presenting with parasitic diseases did not show an increase of CD4 cell surface immunoglobulin after serum incubation with whole blood. No change in the level of surface bound Ig was seen after incubation with high amounts of pooled immunoglobulins.

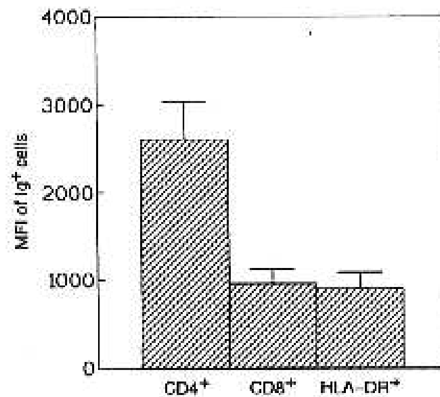

Figure 3. Comparison of surface Ig on different T-cell subsets. The mean fluorescence intensity of Ig^+ T cells was measured in 32 patients (mean+SE). The averages were compared using a paired Wilcoxon signed rank test. $CD4^+$ T cells showed significantly more surface Ig than $CD8^+$ T cells ($P<0.0001$).

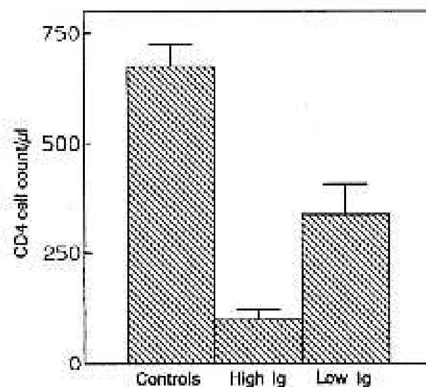

Figure 4. CD4 cell numbers in the blood of patients with high and low levels of surface Ig on $CD4^+$ T cells. CD4 cell counts in controls, patients with low Ig and patients with high Ig on their CD4 cells (mean+SE). Compared with controls patients have significantly lower CD4 cell counts (213 versus 675, $P<0.0001$). Patients with high levels of surface Ig ($n=15$) have significantly lower CD4 cell counts than patients with a lower amount of surface bound Ig ($n=15$) (100 versus 338, $P=0.0063$).

Relationship of increased surface Ig and antibodies against different lymphocyte subpopulations to each other

Highly significant correlations was found between antibodies against $CD4^+$ T cells in the plasma and the mean fluorescence of surface Ig of patients' $CD4^+$ T cells ($r=+0.51$, $P=0.0047$).

Antibodies (plasma) against different T-lymphocyte subpopulations were closely related to each other. Patients with antibodies against $CD4^+$ T cells usually showed antibodies against the other T-cell subpopulations as well. The same was seen for increased surface Ig. The percentages of surface Ig on $CD4^+$ and on $CD8^+$ T cells ($r=+0.95$, $P<0.0001$) and the mean fluorescence intensities of Ig^+ cells ($r=+0.57$, $P=0.0013$) demonstrated a close association. In contrast, the increase of surface Ig on B cells and T-cell subsets seemed not to be related to each other.

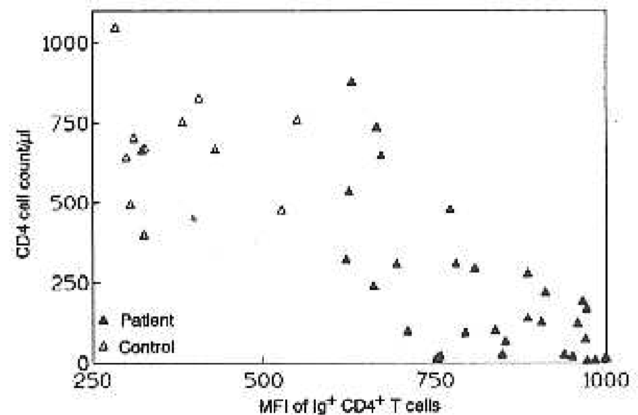

Figure 5. Relationship between surface Ig and CD4 cell depletion. Statistically a highly negative correlation between CD4 cell surface Ig and CD4 cell count was found in HIV-infected patients. The correlation coefficient ($r=-0.67$) is highly significant for $P=0.00005$. The correlation coefficient rises to $r=-0.81$ when controls are included.

Relationship between CD4 cell count and CD4 cell-surface Ig

Patients with high mean fluorescence intensity of Ig^+ $CD4^+$ T cells showed obviously lower CD4 cell numbers than patients with lower levels of surface bound Ig (100 *versus* 338 $CD4^+$ T cells/µl) ($P=0.0063$) (see Fig. 4). Statistical calculations indicated a linear correlation ($r=-0.67$, $P=0.00005$) between increased surface Ig on $CD4^+$ T cells and CD4 cell depletion (see Fig. 5). No significant differences in the number of $CD4^+$ T cells were found comparing the percentages of Ig^+ $CD4^+$ T cells of the patients.

Surface Ig on other T-lymphocyte subpopulations was not associated with CD4 cell depletion, as regards either the percentages or the mean fluorescence intensities of surface Ig on $CD8^+$, $CD3^+$, $HLA\text{-}DR^+$ and $HLA\text{-}DR^-$ T cells.

IL-6

IL-6 concentration was determined in the plasma of patients and controls. IL-6 was detectable (18·5 pg/ml) in the plasma of one HIV-infected patient only and was not detected in control plasma. The HIV^+ patient did not show more surface bound Ig or anti-lymphocyte antibodies in his plasma than other patients but compared to other HIV-infected patients he had markedly elevated serum levels of IgG (24·1 g/l) and IgM (4·83 g/l).

β_2-microglobulin

Furthermore we looked for the relationship of β_2-microglobulin to increased surface Ig and antibodies against lymphocyte subpopulations in the blood of HIV-infected patients and controls. Seventy-seven per cent of the patients showed an elevated plasma level of β_2-microglobulin compared to the values of healthy persons. The average amount of β_2-microglobulin in HIV-infected patients (3400 µg/l) was increased compared to controls (1650 µg/l) ($P=0.002$). Patients with higher serum levels of β_2-microglobulin had lower CD4 cell counts than patients with normal levels of β_2-microglobulin ($r=-0.44$). The immunoglobulin levels in HIV-infected patients tended to be higher in patients with elevated serum β_2-microglobulin concentrations. A relationship between β_2-microglobulin and increased

Table 3. Linear correlation coefficients. A significant negative correlation with the CD4 cell count was observed for β_2-microglobulin. Within the immunoglobulins only one parameter (IgM/% antibodies against CD4 cells) showed a significant association. Antibodies against the viral p24 are slightly correlated with the CD4 cell count in patients

HIV patients ($n=32$)	CD4 cell count (cells/μl)	Plasma anti-CD4 cell antibodies (%)	Increased surface Ig on CD4⁺ T cells (%)
β_2-microglobulin	$r=-0.44^*$	$r=+0.27^{NS}$	$r=+0.29^{NS}$
IL-6	not generally detectable		
IgG	$r=-0.27^{NS}$	$r=+0.30^{NS}$	$r=+0.35^{NS}$
IgM	$r=-0.20^{NS}$	$r=+0.46^{\dagger}$	$r=+0.30^{NS}$
p24 antibodies	$r=+0.20^{NS}$	$r=-0.28^{NS}$	$r=+0.18^{NS}$

$^*P=0.013$; $^{\dagger}P=0.02$; ^{NS} not significant.

surface Ig or antibodies against CD4⁺ T cells (Table 3) was not demonstrated.

Immunoglobulins

The serum levels of IgG ([17·1 ± 6·2 (SD)] and IgM (2·21 ± 1·26 g/l) were raised in patients with HIV infection. Thirty-four per cent of the patients had IgG and 25% had IgM levels above the reference values (IgM 0·6–2·8 g/l; IgG 8–18 g/l). Levels of IgG and IgM were closely related to each other. A slightly negative but not significant correlation was seen between Ig levels and the CD4 cell count in HIV-infected patients (Table 3). Patients with antibodies against CD4⁺ T cells and/or highly increased surface Ig on CD4 cells did not show significantly higher serum levels of IgG and IgM than other patients. We found a positive correlation ($r=+0.46$, $P=0.02$) between the percentage of antibodies against CD4⁺ T cells in the plasma with the serum level of IgM. High correlations were found between β_2-microglobulin and IgG ($r=+0.79$, $P<0.0001$) and IgM ($r=+0.60$, $P=0.006$) serum levels.

Thirty-four per cent of the patients with parasitic diseases displayed elevated levels of IgM (4/13). The mean value was only slightly lower (1·91 ± 1·55 g/l) compared to HIV-infected patients (not significant). Levels of IgG were elevated in a small percentage of patients (2/13).

Antibodies against p24 in the plasma of the patients

Antibodies against p24 were detectable in two-thirds of the patients. Patients with a higher amount of antibodies against p24 usually showed higher numbers of circulating CD4⁺ T cells, but the differences were not significant ($r=+0.20$). Our data did not show any evidence for a relationship to surface bound Ig, soluble immunoglobulins or β_2-microglobulin.

Activated T cells

HIV-infected patients with a higher percentage (> 50%) of HLA-DR⁺ (activated) T cells showed lower CD4 cell numbers than other patients (134 *versus* 303, $P=0.03$). A high percentage of activated T cells in the blood was significantly associated with elevated β_2-microglobulin serum concentrations ($r=+0.49$, $P=0.004$).

Whereas the amount of surface bound Ig on patients' CD4⁺ T cells was not related to a higher degree of cellular activation, the percentage of antibodies against CD4⁺ T cells in the plasma tended to show higher values in patients presenting a higher percentage of activated T cells ($r=+0.43$, $P=0.014$).

DISCUSSION

Our data reveal that more than 90% of HIV-infected patients have highly increased levels of surface bound Ig on CD4⁺ T cells. Other T-cell subsets and B cells showed a lower increase of surface Ig. Out of the controls only one had an increased level of surface bound Ig but the percentage as well as the mean fluorescence intensity was low compared with HIV patients.

These findings diverge from the results of other authors who reported a lower (35–60%) percentage of patients with increased surface Ig on (CD4⁺) T cells.[11-13] The higher amount of anti-human Ig used in our assay could explain the difference. However, lymphocytes of healthy persons did not show an increased level of surface Ig due to the higher amount of antibody used.

In general surface Ig and antibodies against T cells in the plasma were detected by a polyclonal Ig GAM antibody. The use of antibodies specific for IgM and IgG showed that the majority of surface Ig and plasma anti-lymphocyte antibodies are of the IgM and IgG subclasses. In some patients antibodies against IgM reacted more strongly with increased surface Ig than anti-IgG antibodies (data not shown). This confirms previous reports[11] and coincides with anti-lymphocyte antibodies demonstrated in systemic lupus erythematosus and other autoimmune diseases.[21-23]

Daniel and colleagues[11] reported an equal presence of surface Ig on CD4⁺ and CD8⁺ cells. This is in agreement with our results concerning the percentage of Ig⁺ cells in these two subsets. But analysis of the mean fluorescence intensity revealed that CD4⁺ cells had a higher amount of bound Ig.

The origin of the surface Ig on T cells in HIV-infected patients is controversial. Viral RNA and antigen expression are seen in very few cells and cannot be responsible for the increased surface Ig.[24] Also Fc receptor expression is not sufficient to explain this phenomenon, because HLA-DR⁺ T cells (expressing more Fc receptor molecules than non-activated cells) were found to have lower levels of surface bound Ig than CD4⁺ T cells, which are not positive for HLA-DR for the major part.

However, hypergammaglobulinaemia, gp120 bound Ig and autoantibodies may contribute to the widespread phenomenon. To investigate the influence of hypergammaglobulinaemia we

measured IgG and IgM serum levels. We were able to show slightly higher IgG and IgM serum levels in patients with a higher amount of surface Ig. But we did not find significant differences, which is in agreement with a previous report.[13] Weimer and colleagues[10] investigated *in vivo* B-cell activation and autoantibodies (surface Ig) against CD4+ T cells and did not see an association between these parameters.

The linear correlation coefficients between surface Ig and serum IgG and IgM levels were not significant. On the other hand a significant correlation between serum IgM and plasma antibodies against CD4+ cells was demonstrated. This is in contrast to Dorsett and colleagues[20] who did not find such an association. Non-specific Ig deposition on CD4+ T cells, which could be a reason for the positive correlations between plasma anti-CD4 cell antibodies and IgM levels, was not confirmed in our control experiments. Deposition of Ig was neither detectable in patients with hypergammaglobulinaemia due to parasitic diseases nor after incubation of blood with highly concentrated Ig. However, it is possible that the generation of these antibodies (and hypergammaglobulinaemia) is enhanced by polyclonal B-cell stimulation, which is a well-known phenomenon in HIV-infected patients.[25,26] Elevated plasma IL-6 levels seem not to be necessary for the development of increased surface Ig. The majority of our patients including those with increased surface Ig and plasma anti-CD4 cell antibodies did not reveal increased IL-6 plasma levels. These levels are controversial.[27,28] The spontaneous IL-6 production of peripheral blood mononuclear cells (PBMC) was not analysed in our patients, but has been shown to be markedly elevated in HIV-infected patients.[29]

Viral gp120 bound to CD4 cells as a factor of CD4 depletion has been studied by Lyerly and colleagues.[30] They were not able to demonstrate gp120 on the surface of circulating CD4+ T cells. A negative result was reported by Ardman and colleagues[13] as well. We investigated CD4+ T cells of HIV-infected patients for the presence of bound gp120 with a sheep anti-gp120 fluorescence-conjugated antibody (Biochrom, Berlin, Germany). Gp120 on the surface of CD4+ T cells was not detectable in all patients investigated (data not shown).

Autoantibody nature of surface bound Ig has already been discussed by Ardman *et al.*[13] and has been assumed by Daniel *et al.*[11,12] and Weimer *et al.*[10] The majority of HIV-infected patients we have investigated (72%) presented with anti-CD4 cell antibodies in the plasma. Antibodies were not detectable in controls and patients with parasitic diseases. We found a highly significant correlation between surface Ig on patients' CD4+ T cells and anti-CD4 cell antibodies in the plasma of the patients, such an association was reported by Ardman and colleagues as well.[13] This close association leads to the assumption that the majority of increased surface Ig in HIV-infected patients is of autoantibody nature.

Comparison of different serological markers with the CD4 cell count reveals that CD4 cell depletion is associated closer with increased surface Ig on CD4+ T cells than with any other parameter (p24 antibodies, β_2-microglobulin) measured. Patients with high surface Ig had significantly lower CD4 cell counts than patients with low surface Ig. It is speculative whether this association reflects an epiphenomenon, the degree of immune dysregulation or a pathogenetic relevant pathway.

Dorsett and colleagues demonstrated that HIV-infected patients without plasma anti-lymphocyte antibodies did not progress to clinical disease. Ardman showed a close association between CD4 cell-surface Ig and diminished CD4 cell counts in asymptomatic patients only. Together with our own investigations dealing with patients of different stages of disease we find evidence that increased surface Ig (anti-lymphocyte antibodies) might indicate the possibility of a more rapid progression in the disease.

HIV-infected patients usually have an increased amount of activated (HLA-DR+) T cells.[31] The majority of these cells express a cytotoxic phenotype (CD8+ HLA-DR+ CD57+)[32] and autoreactive features have been demonstrated.[33] We were able to show that patients with a higher percentage of HLA-DR+ T cells have significantly lower CD4 cell counts than patients without this phenomenon. Interestingly our data show a significant association between T-cell activation and plasma anti-CD4 cell antibodies ($r = +0{\cdot}43$, $P = 0{\cdot}014$).

In conclusion we have demonstrated that almost all HIV-infected patients have increased surface Ig on their CD4+ lymphocytes. A high percentage of patients have plasma anti-CD4 cell antibodies as well. Both parameters are closely associated. Our data provide evidence for an autoantibody nature of increased surface Ig on CD4+ T cells and this phenomenon is associated with CD4 cell depletion *in vivo*.

Although these data hint at an involvement of antibodies against CD4+ lymphocytes in the pathogenesis of AIDS, origin, function and relevance of these autoantibodies have to be investigated in further studies.

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Polyspecific Self-Reactive Antibodies in Individuals Infected with Human Immunodeficiency Virus Facilitate T Cell Deletion and Inhibit Costimulatory Accessory Cell Function

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Self-reactive polyspecific IgG antibodies (PSAs) arise in human immunodeficiency virus (HIV)–seropositive subjects before they develop AIDS. Self-reactive PSA levels correlate with the destruction of CD8 T cells in HIV-infected individuals and mediate the antibody-dependent cellular toxicity–based destruction of human T cells in tissue culture. PSAs react across the species barrier and bind to T cell antigens in mice. Such reactivity with mouse lymphocytes was not detected in normal human serum. Injection of human PSA IgG causes massive T cell depletion in the spleen, lymph nodes, and thymus in mice: evidence that PSA IgG facilitates T cell destruction in vivo. In addition to facilitating macrophage cytotoxicity, self-reactive PSA IgG inhibits the macrophage-mediated activation of T cells with antigen receptor–specific monoclonal antibody or with antigen. Exogenous costimulatory stimuli or interleukin (IL)-12 can reverse the inhibition. In contrast, exogenous IL-10 mimics this inhibition. These data implicate PSA IgG as a pathogenic factor in the development of HIV disease.

Developing an adaptive immune system, B and T lymphocytes establish reactivity with a diverse universe of antigens by rearranging variable antigen receptor sequences. After leaving the bone marrow, the site of their early maturation, B cells complete their development by executing two major tasks, the immunoglobulin (Ig) class switch and somatic Ig mutation [1]. Ig class switching is induced by cytokines and proceeds independently of antigen receptor (B cell reactivity [BCR]) engagement, whereas somatic mutation is antigen-driven [2, 3].

The BCR rearrangement, which endows B cells with clonotypic receptors, is a random process that results in the formation of receptors reactive with either self-antigens or foreign antigens. Before reaching the stage of functional maturity, each B cell tests its newly made receptors for reactivity with self-antigen and dies when it succeeds [4]. This negative-selection mechanism assures that self-antigens that trigger the BCR into signal transduction destroy the B cell that generated the self-reactive receptor. Self-tolerance is thereby established at a particular trigger threshold of self-reactivity. Self-antigens that react with B cell Ig below that threshold level fail in the negative-selection process and may survive. This must not be cause for concern, because the low-affinity threshold of these self-antigens will not trigger an antigen receptor–mediated immune response of mature B cells. Apart from antigen receptor-mediated activation signals, B cells can be induced to secrete Ig by other stimuli, notably by microbial products [5]. Polyclonally stimulating microbial products react with B-cell surface components distinct from the BCR. They fail to activate the Ig-switching mechanism and do not induce antibody affinity maturation. The switching mechanism is mediated by cytokines, notably interleukin (IL) 4, which are released by helper T cells when they form antigen-specific cellular conjugates with antigen-reactive B cells [6].

It is thus conceivable that nonspecific B cell stimuli can induce B cells to secrete antibodies that may then react with self-antigens that themselves failed to trigger the immune response via the antigen receptor. Unable to engage the antigen receptor, these nonspecific stimuli will not engage B cells in the formation of cellular conjugates with helper T cells and thus neither procure the cytokines needed for Ig class switching nor facilitate antibody affinity maturation. Therefore, nonspecifically activated B cells are not capable of increasing antibody affinities to a given antigen. The antibodies retain low-affinity characteristics with a spectrum of antigens, including self-antigens. Such antibodies are known as polyspecific antibodies (PSAs) [7–9]. Initially secreting IgM, PSA-producing B cells can gen-

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Human experimentation guidelines of the US Department of Health and Human Services and the New York Medical College were followed in the conduct of clinical research.

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erate the whole spectrum of antibody effector functions when they activate the switching mechanism. In the absence of antigen-mediated cellular conjugates with helper T cells, the Ig-IgG switch may proceed in a bystander fashion if the production of switch cytokines occurs for other reasons [2]. These reasons include conditions of chronic pathological immune stimulation seen in long-term systemic exposure to auto-antigen, for example, systemic lupus erythematosus (SLE) [10], and long-term exposure to infectious agents, such as human immunodeficiency virus (HIV) [11,12]. These conditions are characterized by the generation of polyspecific IgG antibodies that react with self-antigen [10-12]. Typically, the T helper (Th) 1/Th2 balance tilts towards a Th2 predominance with enhanced production of the switch cytokines IL-4 and IL-10 [13, 14]. CD5-positive B lymphocytes, which are considered to be major PSA producers, express IL-10 themselves [15]. Herein we investigate whether switching to the IgG class enables lymphocyte-reactive PSAs to disturb immune functions in the host.

Materials and Methods

Study subjects. Serum was prepared from 19 HIV-infected subjects with high levels of polyspecific autoreactive antibodies and from 3 healthy control donors. Six-week-old female Balb/C mice were purchased from the Jackson Laboratories (Bar Harbor, ME). Animals were injected intravenously with human serum (0.1 mL, diluted 1 : 1 in PBS) or purified Ig fractions (600 μg). Lymphoid organs were removed 1-3 days later (as indicated in the data displays), and single-cell suspensions were prepared as described [16]. Lymphocytes were counted and phenotyped in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Experiments were repeated at least twice.

Cell culture. Human peripheral blood mononuclear cells (PBMC) were isolated from donor blood by ficoll-hypaque density sedimentation. Washed cells were resuspended in RPMI 1640 (Sigma, St. Louis, MO) containing 5% fetal calf serum (FCS; Sigma) and counted in an ultraplane-improved Neubauer hemocytometer. Cells at a concentration of 2.5×10^5 were cultured in 0.1 mL RPMI (with 5% FCS) in 96-well, flat-bottom tissue culture plates. PBMC were stimulated with immobilized anti-CD3 monoclonal antibody (mAb) [17]. For immobilization, antibody was added to culture wells at a concentration of 100 μg/mL, incubated overnight at 4°C, and washed 3 times before PBMC was added. An identical immobilization procedure was done with B7-1 Ig and with CD28 mAb. When indicated, *Staphylococcus aureus* enterotoxin B (SEB; Sigma) was added at 1 μg/mL. Other additions are specified in the data displays.

Macrophage-depleted lymphoid cells were prepared by incubating PBMC in RPMI with 20% FCS for 60 min and subsequently gently collecting nonadherent cells. The process was repeated once. The nonadherent cells were cultured alone or were recombined with adherent cells at a ratio of 8 : 1 as described elsewhere [17].

Flow cytometry. Mouse and human lymphoid cells were phenotyped immediately after preparation and after culture by use of a FACScan flow cytometer (Becton Dickinson). Washed cells were stained as described elsewhere [17] with fluorescein-isothiocyanate (FITC)-labeled or biotinylated mAbs. The biotin label was revealed by streptavidin-phycoerythrin (Sigma). The following antibodies were used: anti-mouse CD4 [18], anti-mouse CD8 [19], anti-human CD4 and anti-human CD8 (OKT4 and OKT8, Ortho Diagnostics, Raritan, NJ), and anti-human CD28 (produced by R. Mittler, Bristol-Meyer Squibb, Princeton, NJ). Surface Ig binding was measured by incubating PBMC with PSA-containing human serum (1 : 10 dilution, 4°C, 30 min) followed by treatment with FITC-labeled goat-anti human IgG F(ab)$_2$ (Cappel, West Chester, PA).

Reagents. Human IgG was purified from serum by passage over protein G columns (Sigma) according to the manufacturer's instructions. B7-1 Ig was prepared as described elsewhere [20]. IL-10 was donated by the DNAX Corporation (Palo Alto, CA), and IL-12 was a gift from the Genetics Institute (Boston).

Results

Human PSAs react with human and mouse lymphoid cells and facilitate massive deletion of lymphoid cells in vivo. Human PSAs in the serum of HIV-infected donors and of patients with lupus erythematosus have been shown to bind to human T cells and to enable human macrophages to destroy these T cells in tissue culture [17]. Figure 1 confirms this observation. Serum from an HIV-infected individual and serum from a healthy control

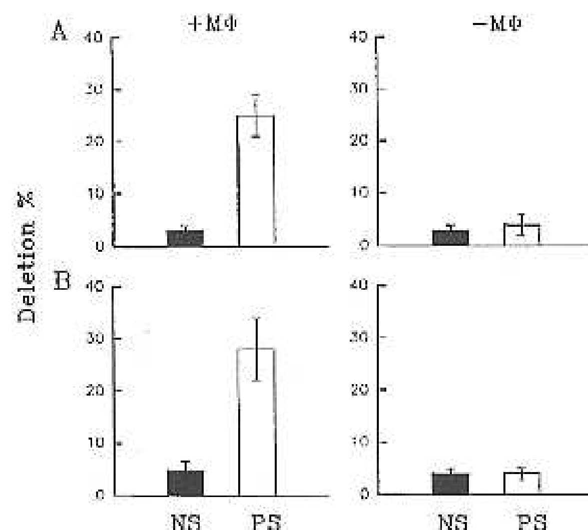

Figure 1. Serum from donors infected with human immunodeficiency virus enables macrophages from seronegative donors to destroy T cells in tissue culture. Human peripheral blood mononuclear nonadherent cells were cultured alone (−MΦ) or in the presence of macrophages (+MΦ), and treated with 1 : 100 dilution of normal serum (NS) or serum from an HIV-infected donor (PS). The cells were harvested 3 days later, counted, phenotyped for the expression of CD4 (*A*) or CD8 (*B*), and assessed for the proportion of deleted cells (percentage) according to the following formula: percentage deleted cells = (cells cultured with NS minus cells cultured with PS)/cells cultured with NS) × 100 [16].

donor were added to freshly prepared human peripheral blood lymphocytes in the presence or absence of macrophages. The cells were harvested 3 days later, counted, and phenotyped for the expression of T cell markers. The experiment shows that macrophages added to lymphocytes in a physiologic ratio destroy lymphocytes in the presence of PSAs but not in their absence. Doubling the monocyte/lymophocyte ratio to that typical of patients with AIDS doubles the fraction of deleted lymphocytes [17]. The observation that self-reactive PSAs facilitate the deletion of T cells in tissue culture has led to the speculation [17] that these antibodies may destroy T cells in vivo and explains the loss of T cells in individuals who express these antibodies. Because these antibodies are self-reactive as a consequence of their polyspecificity, it is possible that they react with lymphocytes from other species. To test this hypothesis, we evaluated antibody-dependent cellular toxicity (ADCC) function of human PSA in mice. Results in figure 2 show that human PSAs cross-react readily with mouse lymphoid cells. Studies were performed injecting serum from HIV-infected subjects into mice. Preliminary results showed that such sera caused rapid and massive depletion of mouse lymphoid cells in blood, lymph nodes, the spleen, and the thymus; serum from healthy donors had no such effect (data not shown). IgG-positive and -negative fractions were prepared from serum of patients with AIDS and administered to mice in comparison to serum from healthy human individuals. Results are shown in figure 3. Approximately one-half of the T cells in spleen and lymph nodes disappear within 1 or 2 days after injection of patient serum IgG. When tested 5 days after injection of human IgG, patient IgG was still found to reduce the frequency of T cells in lymph nodes and spleen. Both single-positive, as well as double-positive, T cells in the thymus declined in frequency (figure 4) [17]. The data confirm the previous results from tissue culture studies and provide evidence that PSA facilitates the massive deletion of lymphocytes in individuals who exhibit them.

Polyspecific self-reactive human antibodies inhibit the receptor-mediated activation of human T cells. Patients with SLE and individuals infected with HIV exhibit both a reduction in T cell counts and diminished or absent T cell responses to mitogenic or antigenic stimuli, reflecting the fundamental immune deficiency in these diseases. To investigate a possible immune-suppressive role of PSAs, we examined whether PSAs affect the response to antigen receptor (T cell reactivity [TCR]) engagement by T cells from healthy donors. Human PBMC were stimulated with CD3 mAb in the presence of IgG from a healthy donor or IgG from an HIV-infected donor. CD3 mAb was immobilized in the culture dish to avoid competition between the mitogenic antibody and human serum IgG fractions for macrophage FcRs. Immobilization renders the TCR stimulus macrophage-FcR–independent [21]. Figure 5*A* shows that patient IgG supports only a fraction of the proliferative mitogen response seen in control cultures and in cultures given IgG from healthy individuals. A similar effect of IgG from patients with AIDS is seen in cultures stimulated with major histocompa-

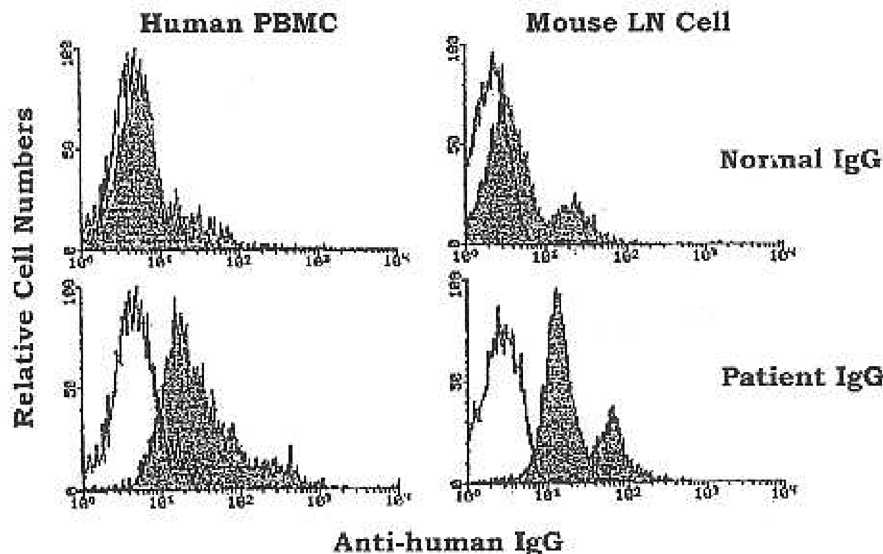

Figure 2. Polyspecific antibodies in the serum of individuals infected with human immunodeficiency virus (HIV) react with autologous lymphoid cells and with mouse lymphocytes. Human peripheral blood mononuclear cells (PBMC) and mouse lymph node cells were incubated for 30 min at room temperature with the IgG fraction of serum from HIV-seronegative (normal IgG) or -seropositive (patient IgG) donors, washed, and counterstained with fluorescein isothiocyanate–labeled goat anti human IgG $F(ab)_2$ antibody. Open-flow cytometer histograms depict anti-IgG reactivity of untreated lymphoid cells; shaded histograms represent treated cells.

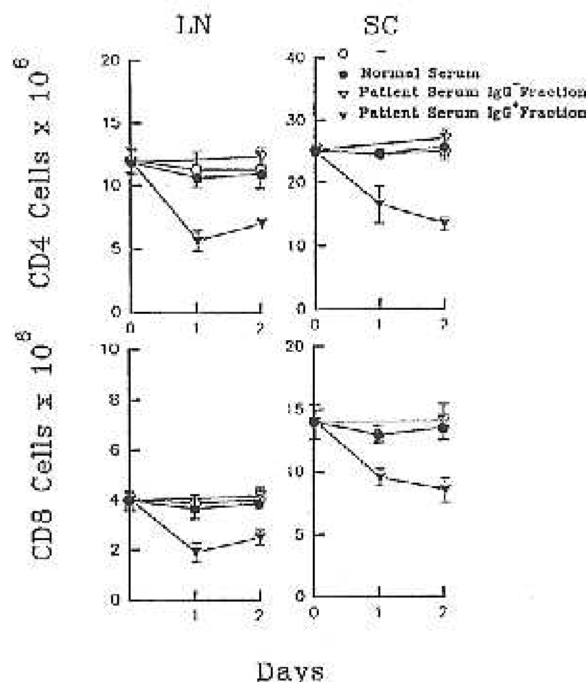

Figure 3. IgG fraction of serum from a patient with AIDS facilitates the deletion of lymphoid cells in Balb/C mice. Balb/C mice were injected with 0.1 mL (1 : 1 diluted with PBS) of normal human serum or the IgG-depleted fraction of serum from patient with AIDS or received an intravenous inoculation with 600 μg IgG from patients with AIDS. Single-cell suspensions were prepared from the spleen or the inguinal lymph nodes 1 or 2 days later, and the cells were counted and phenotyped for coreceptor expression by flow cytometry. The absolute cell counts of several phenotypes were calculated and are shown as the averaged results from 3 animals with SD (error bars). LN, lymph node cells; SC, spleen cells.

tibility complex II–presented superantigen SEB (figure 5*B*). The superantigen recognizes T cells with a restricted number of TCR Vβ families [22], evidently enough to produce a sizable T cell response. The experiments shown in figure 5 present evidence that patients with AIDS produce IgGs that inhibit the immune response of T cells in tissue culture.

It has been suggested that the immune system in HIV-infected subjects lacks appropriate costimulatory capacity [23] and that the defective *in vitro* mitogen response of the T cells of patients with AIDS can be restored by substitution with exogenous costimulatory stimuli [24]. Costimulatory signals are mediated by CD28 molecules on the T cell surface and are induced by B7 family molecules expressed on the surface of accessory cells [25]. We immobilized in the culture vessel 1 of 2 exogenous costimulatory stimuli, soluble B7-1 molecules [20] or CD28 mAb. Both CD28 ligands abrogated the immune suppression induced by PSA IgG (figure 5). These results suggest that IgG from patients with AIDS inhibits the T cell immune response by generating a deficit in costimulatory signals.

Patients with SLE and patients with AIDS have been shown to have an altered cytokine balance, with a shift from a type 1 cytokine predominance (IL-2, interferon-γ, IL-12) to a type 2 cytokine predominance (IL-4, IL-10) [26]. IL-12 and IL-10 are macrophage cytokines whose release has been attributed to 2 distinct macrophage subsets, M1 and M2, respectively [27]. It has been suggested that the M1/M2 ratio declines in HIV-infected subjects [17], which would result in a deficiency of IL-12 production and an excess of IL-10 production. We examined the effects of IL-12 and IL-10 on the immune suppressive activity of IgG from patients with AIDS. We found (figure 6) that IL-12 reverses IgG-mediated immune suppression in vitro of patients with AIDS, whereas IL-10 mimics it. Figure 6 shows an additional assay of the mitogen-induced T cell response in vitro, namely the assessment of T cells that upregulate the expression of CD28 molecules beyond the highest level of resting cells. Fewer T cells respond in this assay in the presence of IgG from patients with AIDS compared with control T cells or T cells treated with normal human IgG. Again, the deficiency is

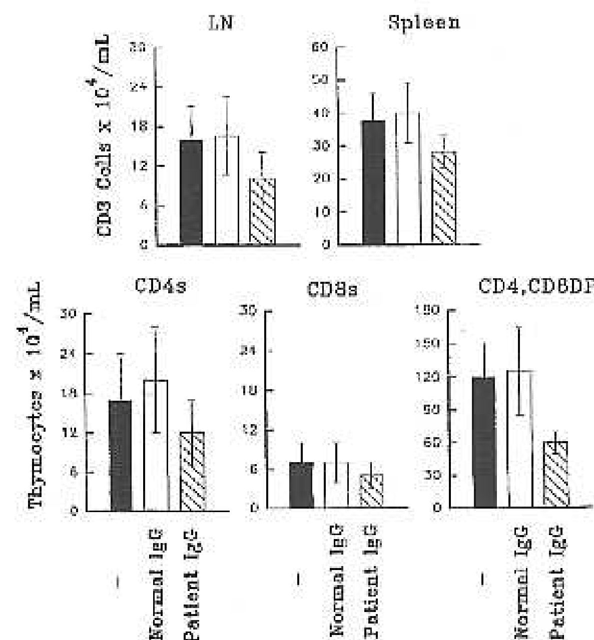

Figure 4. IgG from a patient with AIDS induces a deficiency in the number of mature thymocytes, as well as of immature thymocytes, in the mouse. Balb/C mice were injected with 600 μg IgG from human immunodeficiency virus–seronegative (normal IgG) or –seropositive (patient IgG) donors. Lymphoid organs were recovered 5 days later and phenotyped and evaluated as described in figure 3. The data represent the averaged results of 5 animals ± SD. CD4s and CD8s represent single-coreceptor–positive T cells in the thymus. CD4, CD8DP cells are double-positive immature thymocytes.

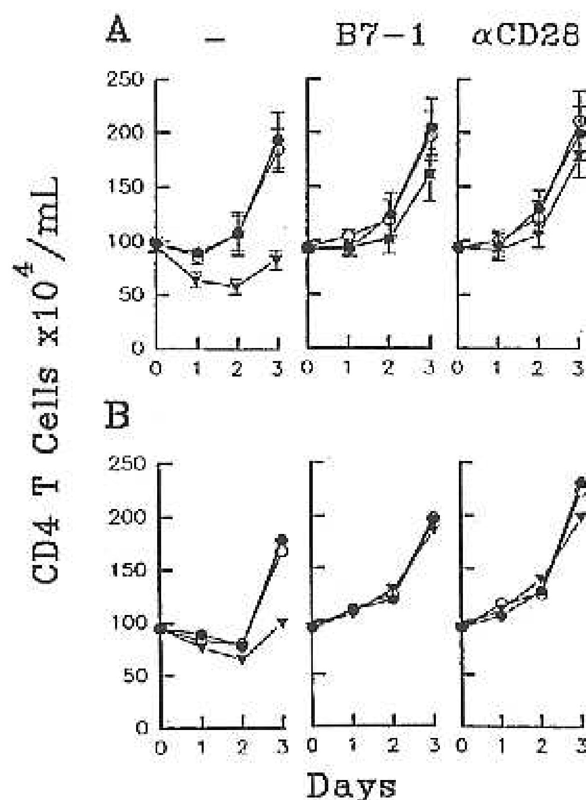

Figure 5. IgG from patients with AIDS containing polyspecific autoreactive antibodies inhibits the receptor-mediated activation of T cells in vitro. Peripheral blood mononuclear cells (PBMC) prepared from a healthy individual were stimulated with a mitogenic T cell reactivity ligand, CD3 monoclonal antibody (*A*), or the superantigen *Staphylococcus aureus* enterotoxin B (*B*) in the absence of human IgG (●) or in the presence of IgG from a donor seronegative to human immunodeficiency virus (HIV; ○) or a donor seropositive to HIV (▲). The cells were harvested on the indicated days. The number of CD4 T cells was calculated. Soluble B7-1 molecules or CD28 mAb were immobilized on the bottom of culture plates prior to the addition of PBMC as indicated on the top of the figure. Error bars represent the SD among 3 experiments. —, no treatment.

corrected in the presence of IL-12 and mimicked in the presence of IL-10. Identical results were obtained with superantigen stimulation of T cells (figure 6*B*).

The concentration of lymphocyte-reactive IgG in serum of patients with AIDS correlates with ADCC and immune-suppressive activity. We wished to determine whether, and to what extent, T cell–reactive antibodies account for immune-suppressive activities of the serum of patients with AIDS. The question was examined in a 2-step procedure. First, we purified IgG from serum of patients with AIDS to isolate antibodies from potentially immune-suppressive serum elements other than antibody. We established the IgG T cell–binding reactivity in serial dilutions and determined for each concentration immune-suppressive and ADCC reactivity values. In a second step, we assayed IgG T cell binding by several unfractionated patient sera and again correlated T cell IgG reactivity with immune-suppressive and ADCC serum reactivity. The results of the experiment are consistent with the interpretation that the immune-suppressive capacity and the ADCC reactivity of unfractionated serum of patients with AIDS is mediated by T cell–reactive IgG and neither enhanced nor suppressed by other serum components.

IgG T cell reactivity was first assessed by incubating fresh PBMC with purified serum IgG and then determining IgG binding in an indirect immunofluorescence assay (figure 7*A*). PBMC were, in addition, treated with the same IgG doses and assayed for ADCC-based T cell depletion and for the ability to proliferate in response to CD3 mAb stimulation. T cell counts were plotted against IgG surface reactivity (anti-human Ig reactivity; figure 7*B*). The results show a good correlation between the amount of PSA IgG bound by the lymphocytes, the induced T cell deletion, and the suppression of the T cell mitogen response.

In the same experiment, we tested 12 unfractionated serum samples from patients with AIDS for levels of lymphocyte-reactive IgG and correlated the intensity of IgG T cell reactivity, measured by indirect fluorescence, with the degree of in vitro immune suppression and ADCC activity (figure 7*C*). Again, the depletion of T cells and the suppression of the mitogen response increased with the amount of IgG bound by the T cells. The regression curves, computer calculated for figures 7*B* and 7*C*, are superposed in figure 7*C* (dashed lines) over those calculated by the computer for the data plotted in figure 7*C* (solid line). The lines show a high degree of correlation and suggest that ADCC reactivity and lymphocyte-related polyreactive antibodies decisively mediate immune suppressive activity of the serum of patients with AIDS.

Discussion

AIDS is an immunologic disease caused by a virus. A hallmark of the disease is a dramatic and initially exclusive decline of CD4 T cells. CD4 T cells play a central role in the regulation of immune functions. Their loss has been regarded as a central cause of the ensuing immune deficiency [28]. This assumption remains popular, despite the fact that experimental depletion of CD4 T cells in humans and animals produced no immune defects comparable to those seen in HIV infection. Extremely low levels of functional CD4 T cells were found to suffice in carrying out basic immune functions [29]. By contrast, T lymphocytes in subjects infected with HIV are compromised in their capacity to carry out immune functions [24, 28, 30]. This is true for CD4 T cells, which are infected by HIV, and for CD8 T cells, which are not infected by HIV [30]. The possibility has been raised that the deficiency is caused by accessory cell dys-

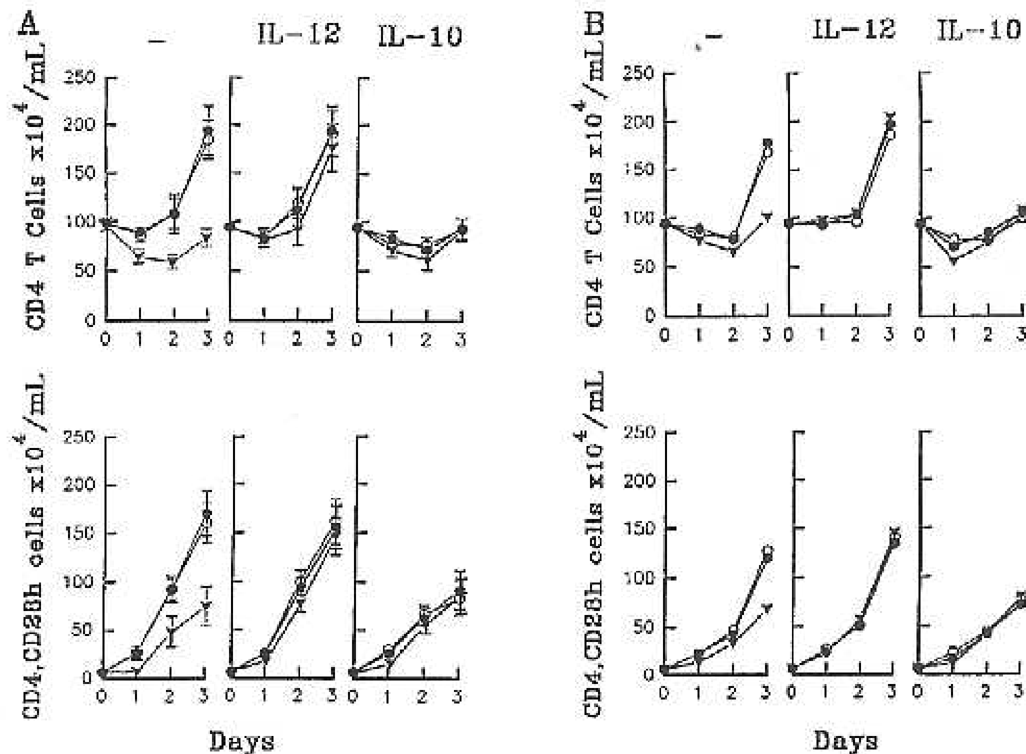

Figure 6. Immune-suppressive effect of IgG from patients with AIDS is reversed by interleukin (IL)-12 and mimicked by IL-10. Peripheral blood mononuclear cells prepared from healthy individuals were stimulated with immobilized anti-CD3 monoclonal antibody (*A*) or *Staphylococcus aureus* enterotoxin B (*B*) in the presence of 100 U/mL IL-12 or 30 ng/mL IL-10 or in the absence of cytokines. The number of CD4 T cells was determined in the upper panels; the number of CD4 T cells that upregulated the expression of CD28 (CD28h) as a consequence of receptor-mediated stimulation was determined in the lower panels. Error bars represent the SD among 3 experiments. —, no cytokine added.

function, namely by their inability to generate appropriate costimulatory signals [23]. It has been shown that macrophages in HIV-infected donors lose the capacity to present specific recall antigens [31].

Controversy persists regarding the mechanism of CD4 T cell depletion. The virologic view is that HIV infects CD4 T cells and accounts for their direct destruction. However, immunologic studies have revealed that among the masses of dying T cells, only a small fraction is HIV-infected [32]. As an alternative explanation, it has been suggested that CD4 T cells are destroyed by indirect means. It has been shown that the CD4-reactive HIV envelope molecule gp120 forms gp120/IgG immune complexes that mediate the ADCC-based destruction of CD4 T cells [17, 23, 33, 34]. Treatment of human PBMC cultures with gp120/IgG causes the destruction of CD4 T cells [17, 23, 33, 34], and injection of such immune complexes in CD4^{hu}-transgenic mice deletes T cells that exhibit the human marker [35].

Recent studies emphasize a critical role of HIV-infected macrophages in the pathogenesis of AIDS [17]. It has been known for some time that chimpanzees replicate T-tropic HIV variants readily but not M-tropic variants and that chimpanzees infected with HIV do not develop AIDS [36]. Humans with a genetic deficiency in replicating M-tropic HIV variants are also resistant to HIV disease [37]. HIV infection alters macrophage functions fundamentally. It inhibits macrophage costimulatory activities [17, 23, 24, 26] and enhances macrophage cytotoxic activities in the ADCC reaction [17, 23, 33, 34]. Two distinct macrophage subsets have been proposed, a costimulatory set that disappears after HIV infection and a cytotoxic subset that expands after HIV infection [17, 23, 27]. The biologic function of lymphocyte-reactive PSAs should be considered in the context of macrophage functions, namely in the context of costimulation and cytotoxicity. We have previously shown, and confirm here, that lymphocyte-reactive PSAs facilitate the deletion of CD4 and CD8 T cells by cytotoxic macrophages [17]. Reacting with T cell surface components, PSAs target T cells for ADCC-based destruction by macrophages or other cytotoxic cells with ADCC activity. Furthermore, our data show that PSAs generate a deficit of macrophage costimulatory activity

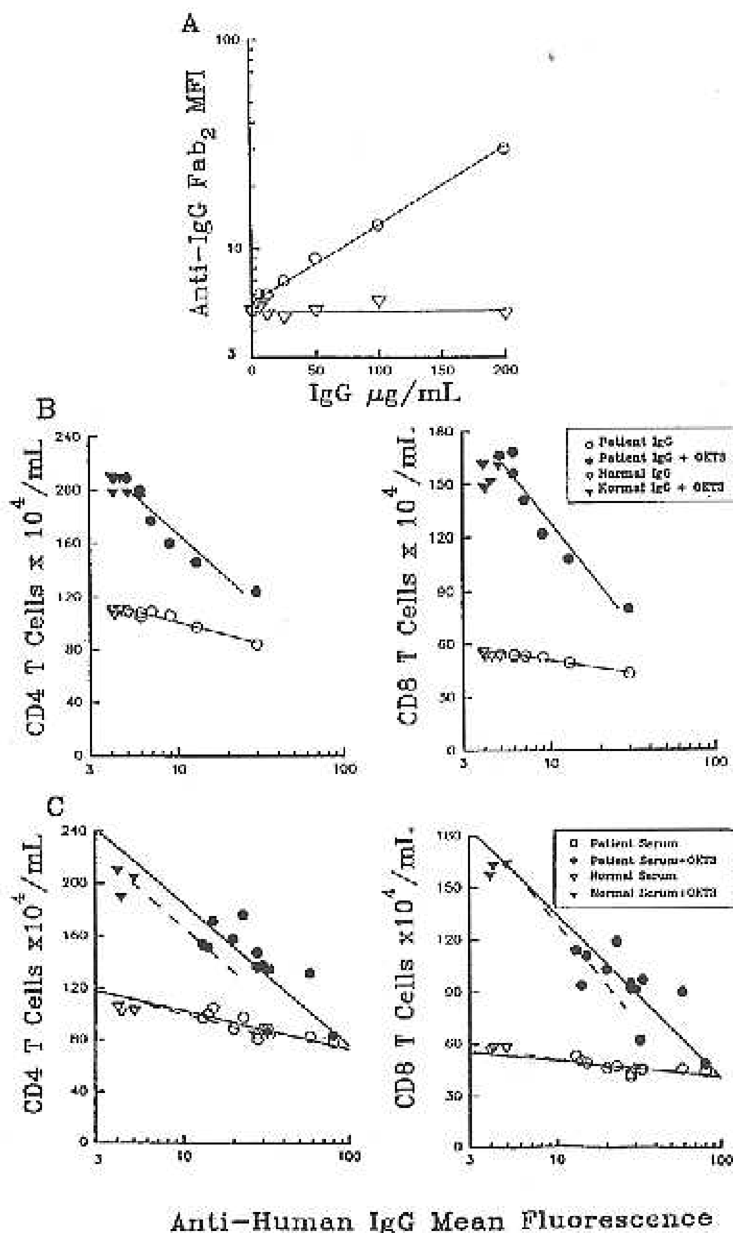

Figure 7. Concentration of lymphocyte-reactive IgG in the serum of patients with AIDS correlates with its antibody-dependent cellular toxicity activity and immune suppressive action. *A*, human peripheral blood mononuclear cells incubated with different concentrations of IgG from human immunodeficiency virus–seronegative (△) or –seropositive (○) donors. The self-reactivity was measured in the flow cytometer by indirect immunofluorescence by using fluorescein isothiocyanate–conjugated anti-human IgG F(ab)$_2$. *B*, T cell reactivity (anti-IgG fluorescence, abscissa) of the same IgG doses correlated with the effect on T cell survival (open symbols) and proliferation in response to CD3 monoclonal antibody (filled symbols). Regression lines were drawn by the computer. Unfractionated sera from 3 healthy individuals and from 12 patients with AIDS were tested in (*C*) for T cell reactivity (anti-IgG fluorescence, *x* axis) and for their effects on T cell survival (open symbols) and mitogen responsiveness (filled symbols). Regression lines were drawn by the computer, but the regression lines from (*B*) were transferred by hand (dashed lines) to examine the fit of the 2 lines.

that can be reversed when exogenous costimulatory factors are administered. Self-reactive PSAs may thus contribute to both the depletion of T cells and to the impaired immune response in HIV-infected subjects.

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Human Immunodeficiency Virus Type 1–infected Individuals Make Autoantibodies that Bind to CD43 on Normal Thymic Lymphocytes

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Summary

Sera from human immunodeficiency virus type 1 (HIV-1)-infected and -noninfected individuals were screened for antibodies that could bind to native T cell differentiation antigens. Antibodies that could immunoprecipitate CD43 (sialophorin, leukosialin) from a T cell lymphoma line were detected in sera from 27% of patients, and antibodies that could bind specifically to transfected cells expressing CD43 were detected in 47% of patients. The anti-CD43 antibodies were related to HIV-1 infection in that no patients with other chronic viral infections or systemic lupus erythematosus contained such antibodies in their sera. The anti-CD43 autoantibodies bound to a partially sialylated form of CD43 expressed by normal human thymocytes, but not by normal, circulating T lymphocytes. However, the determinant(s) recognized by the anti-CD43 autoantibodies was present on a large proportion of circulating T lymphocytes, but masked from antibody recognition by sialic acid residues. These results demonstrate that HIV-1 infection is specifically associated with the production of autoantibodies that bind to a native T cell surface antigen.

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The severe immunodeficiency associated with HIV-1 infection is believed to result primarily from depletion, and to some degree, dysfunction of CD4+ T lymphocytes (1). Many individuals infected by HIV-1 have serum anti-T lymphocyte antibodies (2–9), and it has been suggested that such antibodies contribute to the CD4+ lymphocyte abnormalities of AIDS. Antilymphocyte antibodies that mediate complement-induced lysis of allogeneic T cells (2–4, 7, 8) or suppress mitogen-induced proliferation of allogeneic CD4+ lymphocytes (8) have been detected in sera from HIV-1-infected individuals. Moreover, circulating T lymphocytes coated by autologous Ig (surface Ig+ T cells) have been detected in a substantial proportion of infected patients (10–12). Such surface Ig+ T cells can appear early in the course of HIV-1 infection, before decrements in the absolute CD4+ lymphocytes counts (12).

Despite the potential immunosuppressive effects of antilymphocyte antibodies, their actual contribution to the immunopathogenesis of AIDS remains controversial. One problem has been the difficulty in identifying specific T cell surface antigens recognized by such antibodies. Although serum antibodies that bind to soluble, recombinant forms of CD4 (sCD4) appear specific for HIV-1 infection, (13–15), they do not bind to native CD4 on lymphocytes (13). Other lymphocyte-derived proteins that react with antibodies from HIV-1+ sera (8, 16) have been described, but the identities of these proteins and their cellular location (i.e., cell surface vs. intracellular) remain uncertain.

In the present study, we show that many HIV-1-infected individuals make autoantibodies that bind specifically to the T cell surface antigen CD43. CD43, also known as sialophorin (17) or leukosialin (18), is a heavily sialylated glycoprotein expressed on the surface of virtually all thymocytes and T lymphocytes (19, 20). The anti-CD43 autoantibodies from HIV-1-infected individuals recognize a partially sialylated form of CD43 that is present on normal human thymocytes but not on mature, circulating T lymphocytes. The potential significance of these results is discussed in the context of recent insights into the role of CD43 in immune physiology.

Materials and Methods

Cells and Cell Lines. Normal human PBMC were obtained from heparinized blood samples from healthy donors and prepared by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density centrifugation. PBMC were treated with 5 μg/ml of PHA (Sigma Chemical Co., St. Louis, MO) for 3 d and then with 20 U/ml human rIl-2 (Cetus Corp., Emeryville, CA) for 5 d to obtain T lymphoblasts. Human thymocytes were obtained from infants undergoing corrective cardiac surgery at the New England Medical Center Hospital. The T cell lymphomas cell line SupT1 (21)

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was provided by Dr. James Hoxie, University of Pennsylvania, Philadelphia, PA. The T lymphoblasts and the SupT1 cell line were grown in RPMI 1640 media (containing 10% FCS [Hyclone Laboratories, Logan, UT] supplemented with glutamine [2 mM], penicillin [100 U/ml], and streptomycin [100 μg/ml]). COS cells used for transfection studies were maintained in similar media, substituting gentamicin sulfate (50 μg/ml) for penicillin and streptomycin.

Sera and mAbs. Sera from HIV-1-infected individuals was provided by the Boston City Hospital Immunodeficiency Clinic and the Fenway Community Health Center, Boston, MA; from hepatitis B antigenic (HBsAg$^+$)[1] individuals (Dr. Barbara Werner, Massachusetts State Laboratory, Boston, MA); from HTLV-1-seropositive individuals (Dr. Antonella Caputo, Dana Farber Cancer Institute, Boston, MA); and from patients with SLE (Dr. Paul Demchak, New England Center Hospital, Boston, MA). Anti-CD1 mAb (OKT6 ascites) was a gift from Dr. Judith Swack, New England Medical Center Hospital. Anti-intracellular adhesion molecule type 1 (ICAM-1) mAb (RR1/1) was provided by Dr. Timothy Springer, Center for Blood Research, Boston, MA. Anti-Leu-22 (CD43) and anti-Leu-3a (CD4) were purchased from Becton Dickinson & Co. (Mountain View, CA). PE-conjugated OKT3 (CD3) was purchased from Ortho Diagnostic Systems, Inc., Westwood, MA.

Preparation of Antibody-containing Eluates. The eluates were prepared by incubating 1 ml of human serum (diluted 1:40 in PBS, pH 7.2, containing 1% BSA and 0.2% sodium azide) with 10^7 SupT1 cells for 2 h at 4°C. The cells were washed four times with 40 ml of ice-cold PBS, and the bound antibodies were eluted using 2 ml of 0.1 M glycine, pH 2.5, for 2 min on ice. The cells were removed by centrifugation (1,000 g), the eluates were neutralized with 200 μl of 1 M Tris, pH 8.0, and then dialyzed extensively against PBS, pH 7.2, before use. Antibody concentration of the eluates was determined by ELISA.

Immunofluorescence. For staining SupT1 cells, sera clarified by centrifugation (10,000 g for 10 min) were diluted 1:50 in PBS containing 1% BSA and 0.2% sodium azide (staining buffer) and incubated for 30 min on ice with the SupT1 cells. After washing in staining buffer, the cells were incubated for 30 min on ice with FITC-conjugated goat anti-human IgG $F(ab')_2$ (Tago Inc., Burlingame, CA) diluted 1:60 in staining buffer. For staining thymocytes, 1 μg/ml anti-Leu-22 or 0.25 μg/ml of human antibody-containing eluate was used, diluted in staining buffer. Binding of anti-Leu-22 was detected by FITC-conjugated goat anti-mouse IgG $F(ab')_2$ (Tago Inc.), and binding of IgG from the eluates was detected by the goat anti-human IgG reagent described above. After staining, all cells were washed in staining buffer, fixed in 1% formalin, and analyzed on an Epics 541 flow cytometer (Coulter Electronics Inc., Hialeah, FL).

Radiolabeling. SupT1 cells (2×10^7) or 4×10^7 thymocytes or 5×10^6 COS cells were washed four times in PBS and resuspended in 1 ml of PBS containing 10 U of bovine milk lactoperoxidase (Calbiochem-Behring Corp., San Diego, CA) and 1 mCi of $Na^{125}I$ (Amersham Corp., Arlington Heights, IL). Then, 25 μl of 0.03% hydrogen peroxide was added to the cell suspension initially and at three successive times at 5-min intervals. Sodium azide (25 μl of a 20% solution) was added to the cell suspensions, the cells were washed four times in PBS, and then solubilized in lysis buffer (0.05 M Tris buffer, pH 7.2, containing 1% Triton X-100 (Sigma Chemical Co.) and 1 mM PMSF. The cell lysates were clarified by centrifugation (12,000 g for 30 min), and the supernatants were used for immunoprecipitations.

Immunoprecipitation and Gel Electrophoresis. For immunoprecipitation of radiolabeled cell lysates, aliquots were precleared with protein A–Sepharose 6MB beads (Pharmacia Fine Chemicals) and then mixed with antibody (1 μg of mAb or 0.25 of eluate) that was prebound to 50 μl of packed beads. The mixtures were incubated for 12–18 h at 4°C, the beads were washed six times with lysis buffer, and the immune complexes were eluted by heating for 5 min at 80°C in 2× sample buffer containing 50 mM Tris, pH 6.8, 4% SDS, 2% glycerol, 10% 2-ME and 0.02% bromphenol blue dye. The immunoprecipitates were resolved by 10% SDS-PAGE, and the dried gels were autoradiographed at −70°C for 5 d using an intensifying screen. To prepare samples for the immunoblotting experiment, 6×10^7 SupT1 cells were solubilized in lysis buffer, the lysates were clarified by centrifugation, and then precleared with protein A beads. Equal volume aliquots of the lysates were immunoprecipitated by anti-Leu-22 (1 μg), anti-Leu-3a (1 μg), or pooled eluate (0.25 μg) prebound to protein A beads (50 μl). The beads were washed six times in lysis buffer, the immune complexes were eluted by heating in 2× sample buffer, and were resolved by 10% SDS-PAGE.

Immunoblotting. Immunoprecipitates of unlabeled SupT1 cells resolved by SDS-PAGE were blotted onto nitrocellulose paper as described (22). The nitrocellulose paper was blocked in 0.05 M Tris-buffered saline, pH 7.0, containing 5% nonfat dry milk (blocking buffer), and then reacted for 18 h at 4°C with anti-Leu-22 (1 μg/ml), diluted in blocking buffer. The blot was washed extensively in blocking buffer and then reacted with alkaline phosphatase–conjugated goat anti-mouse Ig (Boehringer Mannheim Biochemicals, Indianapolis, IN) diluted 1:600 in blocking buffer for 2 h at room temperature. After further extensive washing, the blot was developed with a precipitating substrate (23).

Glycosidase Treatment of Cells and Immunoprecipitates. Neuraminidase treatment of cells was performed in RPMI 1640 with 0.1 U/ml of enzyme at 37°C for 30 min. Enzyme digestions of immunoprecipitates were performed as follows: *Vibrio cholera* neuraminidase (Calbiochem-Behring Corp.), 0.1 μ/ml for 60 min at 37°C; O-Glycanase (Genzyme, Boston, MA), 4 mU/ml for 18 h at 37°C. The enzyme reaction mixtures contained 0.17% SDS, 0.3% 2-ME, 1.25% NP-40, 5 mM calcium carbonate, 10 mM phenanthroline, and 20 mM sodium cacodylate, pH 6.5.

Plasmid Construction and Cell Transfection. The PEER-3 cDNA clone of CD43 (provided by Dr. Minoru Fukuda, La Jolla, CA [24]) was subcloned into an expression vector (CDM8) that utilizes the cytomegalovirus early promoter and contains simian virus 40 origin of replication (25). The 1.5-kb PEER-3 cDNA was isolated from Bluescript by digestion with EcoRI and low melting point agarose gel electrophoresis. The recovered cDNA was blunt ended using the Klenow fragment of DNA polymerase I, ligated to BstX1 linkers and to BstX1-digested CDM8. A DEAE-dextran method (25) was used to transfect COS cells with the CD43-CDM8 plasmid.

Results

Detection of Anti-T Lymphocyte Antibodies in HIV-1$^+$ Sera. To determine if antibodies to native T lymphocyte surface antigens are produced by HIV-1-infected individuals, we screened sera by immunofluorescent flow cytometry to identify those containing antibodies that could bind to a T cell

[1] *Abbreviations used in this paper:* HBsAg$^+$, hepatitis B antigenemic; HTLV-1, human T lymphocyte virus type 1; ICAM-1, intracellular adhesion molecule type 1; WA, Wiskott-Aldrich.

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lymphoma line (termed SupT1). This cell line was chosen because it expresses multiple T cell antigens including high levels of CD4 (26). Sera from several HIV-1-seropositive individuals contained antibodies that stained the SupT1 cell line (Fig. 1). In contrast, no sera from hepatitis B–antigenemic individuals ($HBsAg^+$) and few sera from individuals seropositive for human T lymphocyte virus type 1 (HTLV-1) infection or with the autoimmune disease SLE demonstrated staining of SupT1 cells greater than sera from healthy, HIV-1-seronegative control subjects (Fig. 1).

Identification of Cell Surface Protein Recognized by Anti-T Lymphocyte Antibodies. To identify the SupT1 surface antigen(s) recognized by the serum antibodies, sera were absorbed to SupT1 cells, and the eluted antibodies (termed eluates) were used to immunoprecipitate detergent-solubilized lysates from surface ^{125}I-labeled SupT1 cells. Of 18 eluates prepared from different HIV-1$^+$ sera (sera also positive for SupT1 staining), eight immunoprecipitated a single SupT1 surface protein with a M_r of 120 kD on SDS-PAGE (Fig. 2 *a*). The 120-kD protein was not immunoprecipitated by eluates prepared from the two HTLV-1$^+$ sera and the one SLE serum that did stain the SupT1 cells. Thus, antibodies that immunoprecipitated the 120-kD SupT1 protein were detected only in HIV-1$^+$ sera.

Biochemical Characterization of 120-kD SupT1 Protein. To assess the contribution of carbohydrate to the 120-kD SupT1 protein, immunoprecipitates were subjected to glycosidase digestion. Neuraminidase digestion of the immunoprecipitated 120-kD SupT1 protein shifted its M_r to ~150 kD (Fig. 2 *b*, lane *N*), a result consistent with removal of negatively charged sialic acid residues (18, 19). Subsequent *O*-Glycanase digestion of the neuraminidase-treated immunoprecipitate resulted in a M_r of ~110 kD, indicating the presence of *O*-linked oligosaccharides (Fig. 2 *b*, lane *N/O*). Treatment of the immunoprecipitated 120-kD protein with *N*-Glycanase did not result in a discernable shift in relative molecular mass of the protein (data not shown), suggesting that little or no *N*-linked oligosaccharides were present. These biochemical characteristics suggested that the 120-kD SupT1 protein was similar to CD43 (sialophorin, leukosialin), a cell surface sialoglycoprotein predominantly expressed by cells of hematopoietic origin (19, 20).

The 120-kD SupT1 Protein Is Identical to CD43. The results of two experiments established that the 120-kD protein immunoprecipitated by the eluates is CD43. First, an anti-CD43 mAb (anti-Leu-22; clone L60) (27) bound to the 120-kD SupT1 protein that was immunoprecipitated by pooled eluates, and then electroblotted onto nitrocellulose paper (Fig. 2 *c*). Second, the eluates immunoprecipitated a 130-kD protein from COS cells transfected with a cDNA clone encoding CD43 (Fig. 2 *d*, lane *A*) but not from control COS cells transfected with a cDNA clone of ICAM-1 (Fig. 2 *d*, lane *B*). anti-Leu-22 specifically immunoprecipitated a protein with a M_r of 120 kD from the COS cells expressing CD43 (Fig. 2 *d*, lane *C*), suggesting that in transfected COS cells, anti-Leu-22 recognizes a more sialylated form of CD43 than that recognized by the eluates.

Anti-CD43 Autoantibodies Bind Only to CD43 Expressed by Thymocytes. To determine if the eluates could bind to normal human cells of T cell lineage known to express CD43, two eluates (from two different HIV-1-infected subjects) containing anti-CD43 antibodies were tested for binding to thymocytes, fresh peripheral blood T lymphocytes, and PHA-activated T lymphocytes that had been maintained in IL-2. The eluates bound only to thymocytes, whereas the anti-CD43 mAb (anti-Leu-22) bound to all three cell types (Fig. 3 *a*). However, the eluates bound to all cell types if the cells were first treated with neuraminidase, a treatment that eliminated the epitope recognized by anti-Leu-22 (Fig. 3 *b*). These results suggested that the eluates recognize a non-sialic acid epitope of CD43 present on both thymocytes and mature T lymphocytes, but accessible to autoantibody binding only on thymocytes.

The eluates were also tested for binding to freshly obtained peripheral blood T lymphocytes and T cell lines from HIV-

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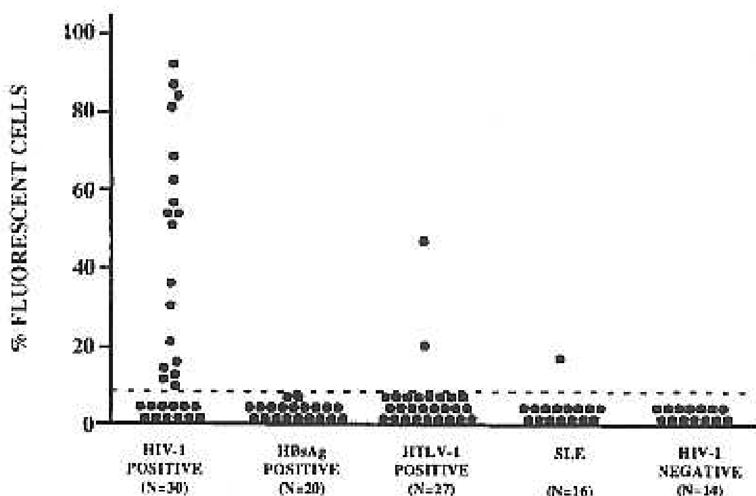

Figure 1. Immunofluorescent screening of sera for antibodies that could bind to the SupT1 T cell lymphoma line. Percent fluorescent cells indicates the percentage of cells stained by each serum (diluted 1:50) greater than stained by buffer alone. Each dot represents the percentage of cells stained by an individual serum. The horizontal dotted line indicates 3 SD above the mean percentage of cells stained by sera from 14 healthy, individually tested, HIV-1 seronegative laboratory personnel.

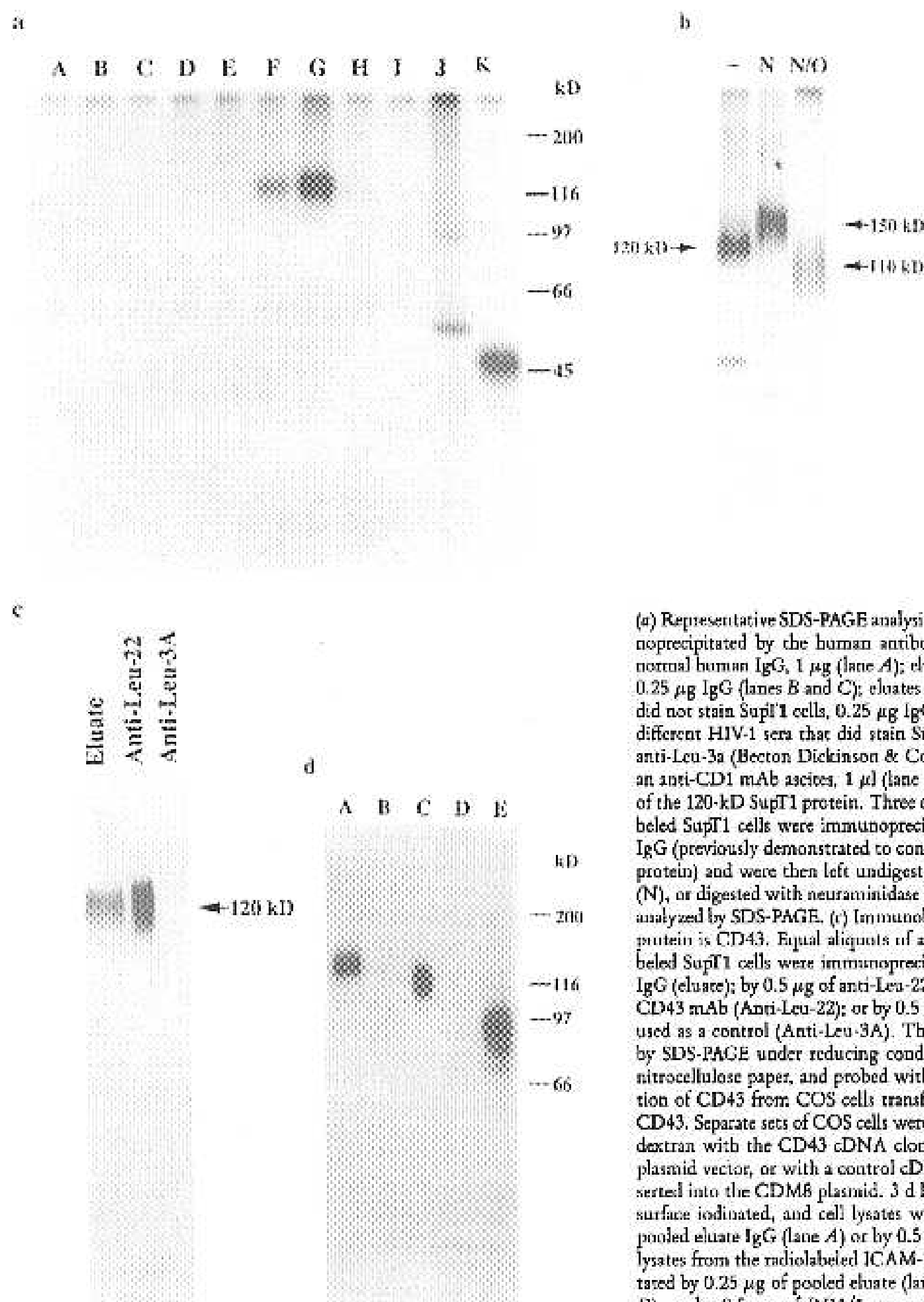

Figure 2. Identification and characterization of the SupT1 cell surface protein recognized by the human antibody–containing eluates. (*a*) Representative SDS-PAGE analysis of the SupT1 surface protein immunoprecipitated by the human antibody-containing eluates. Antibodies: normal human IgG, 1 μg (lane *A*); eluates from two different HIV-1$^-$ sera, 0.25 μg IgG (lanes *B* and *C*); eluates from two different HIV-1$^+$ sera that did not stain SupT1 cells, 0.25 μg IgG (lanes *D* and *E*); eluates from four different HIV-1 sera that did stain SupT1 cells, 0.25 μg IgG (lanes *F–I*); anti-Leu-3a (Becton Dickinson & Co.) an anti-CD4 mAb, 1 μg (lane *J*); an anti-CD1 mAb ascites, 1 μl (lane *K*). (*b*) Biochemical characterization of the 120-kD SupT1 protein. Three equal aliquots of lysates from radiolabeled SupT1 cells were immunoprecipitated by 0.25 μg of pooled eluate IgG (previously demonstrated to contain antibodies to the 120-kD SupT1 protein) and were then left undigested (−), digested with neuraminidase (N), or digested with neuraminidase followed by O-Glycanase (N/O) and analyzed by SDS-PAGE. (*c*) Immunoblot evidence that the 120-kD SupT1 protein is CD43. Equal aliquots of a lysate prepared from 6 × 10^7 unlabeled SupT1 cells were immunoprecipitated by 0.25 μg of pooled eluate IgG (eluate); by 0.5 μg of anti-Leu-22 (Becton Dickinson & Co.), an anti-CD43 mAb (Anti-Leu-22); or by 0.5 μg of anti-Leu-3a, an anti-CD4 mAb used as a control (Anti-Leu-3A). The immunoprecipitates were resolved by SDS-PAGE under reducing conditions, electroblotted onto 0.45-μm nitrocellulose paper, and probed with anti-Leu-22. (*d*) Immunoprecipitation of CD43 from COS cells transfected with a cDNA clone encoding CD43. Separate sets of COS cells were transiently transfected using DEAE-dextran with the CD43 cDNA clone PEER-3 inserted into the CDM8 plasmid vector, or with a control cDNA encoding ICAM-1 (43), also inserted into the CDM8 plasmid. 3 d later, each set of transfected cells was surface iodinated, and cell lysates were immunoprecipitated by 0.25 μg pooled eluate IgG (lane *A*) or by 0.5 μg of anti-Leu-22 (lane *C*). Control lysates from the radiolabeled ICAM-1 transfectants were immunoprecipitated by 0.25 μg of pooled eluate (lane *B*), by 0.5 μg of anti-Leu-22 (lane *D*), or by 0.5 μg of RR1/1, an anti-ICAM-1 mAb (lane *E*).

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1-seropositive individuals (n = 3). In all cases, the eluates bound only to neuraminidase-treated cells (data not shown). These results suggest that peripheral blood T lymphocytes from HIV-1-infected individuals either do not express partially sialylated forms of CD43, or if they do, are targeted for rapid removal from the circulation.

Immunoprecipitation of cell lysates from radiolabeled thymocytes confirmed that the anti-CD43 autoantibodies recognize a partially sialylated form of CD43. The pooled eluates immunoprecipitated a single protein with a M_r of ~130 kD, whereas anti-Leu-22 immunoprecipitated a protein with a M_r of ~120 kD (Fig. 3 *c*, lanes *A* and *B*). However, after digestion of each immunoprecipitate with neuraminidase, the proteins immunoprecipitated by the pooled

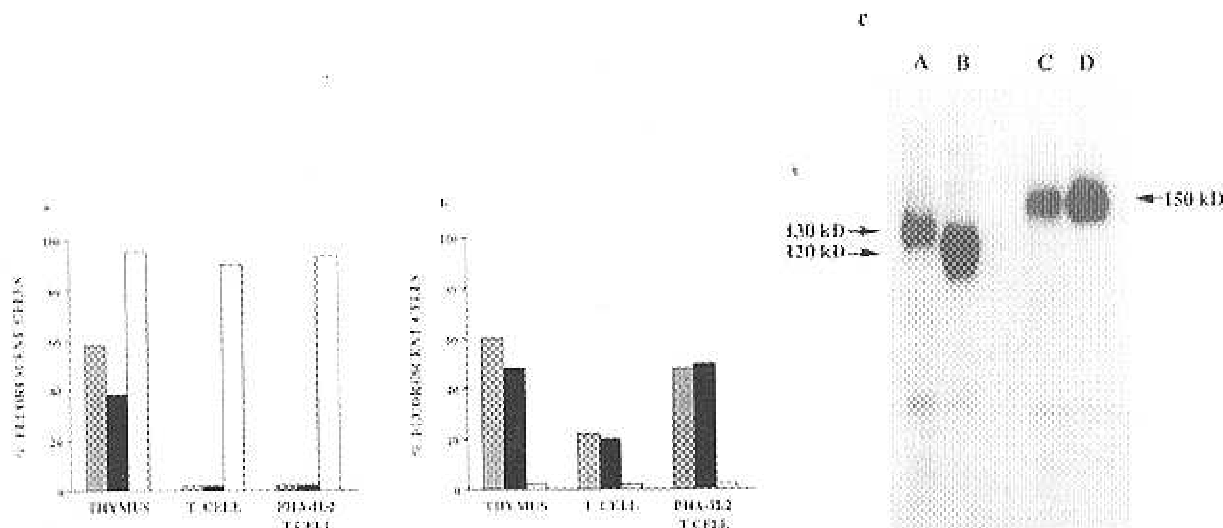

Figure 3. The eluates identify a partially sialylated CD43 form expressed by normal thymocytes. (*a*) The eluates bind to thymocytes but not T lymphocytes. Eluates from patients M-1 (*shaded bars*) and E-1 (*black bars*), and anti-Leu-22 (*white bars*) were tested by indirect immunofluorescence for binding to thymocytes (thymus), peripheral blood T lymphocytes (T cells), and T lymphocytes stimulated with PHA for 3 d and maintained in IL-2 for 7 d (PHA-IL-2 T cells). The results are representative of three separate experiments in which thymocytes and T lymphocytes from other donors were tested. (*b*) The eluates bind to all cell types after cells are treated with neuraminidase (0.1 U/ml in RPMI 1640 for 30 min at 37°C), but reactivity of anti-Leu-22 is lost. (*c*) Immunoprecipitation of CD43 from normal human thymocytes by the pooled eluates (lanes *A* and *C*) and anti-Leu-22 (lanes *B* and *D*). The immunoprecipitates in lanes *C* and *D* were treated with neuraminidase (0.1 U/ml) before electrophoresis. 0.5 μg of pooled eluates or 0.5 μg of anti-Leu-22 were used to immunoprecipitate equal aliquots of ^{125}I-labeled cell lysate from 4 × 10^7 normal thymocytes.

eluates, and anti-Leu-22 migrated identically with a M_r of 150 kD (Fig. 3 *c*, lanes *C* and *D*). These results are consistent with those from the COS cell transfection experiments where two forms of CD43 were identified (Fig. 2 *d*), differing only in their degrees of sialylation. Taken together with the immunofluorescence results, the data confirm that a partially sialylated CD43 form is normally expressed on a large subpopulation of thymocytes but not on mature T lymphocytes (19).

Discussion

These studies demonstrate that anti-CD43 autoantibodies can be detected in sera from HIV-1 infected individuals and that these autoantibodies bind to a form of CD43 expressed by normal thymocytes. The results also indicate that the autoantibodies recognize a CD43 epitope(s) on circulating T lymphocytes that is masked by sialic acid residues. These characteristics distinguish the anti-CD43 autoantibodies from antilymphocyte antibodies described previously (2–9) that bind circulating lymphocytes. The absence of partially sialylated CD43 forms on circulating lymphocytes (19) suggests that the anti-CD43 autoantibodies we detected could not contribute to depletion of circulating CD4$^+$/CD43$^+$ lymphocytes. Rather, the thymocyte specificity of these antibodies suggests that they may interfere with replenishment of the circulating lymphocyte pool. Because anti-CD43 autoantibodies were found only in HIV-1-infected individuals, it is possible that they are involved in the immunopathogenesis of AIDS.

It has been demonstrated that binding of mAbs to CD43 can induce biochemical and functional changes in T cells in vitro. These antibody-mediated effects include induction of phosphoinositide hydrolysis with resultant second messenger formation (28); homotypic adhesion of lymphocytes (29) and monocytes (30); and activation of thymocytes (31) and T lymphocytes (29, 32) by a mechanism independent of TCR/CD3 complex–mediated signaling (28). It is plausible that the effects mediated by anti-CD43 mAbs in vitro would be mirrored by human anti-CD43 autoantibodies in vivo, resulting in inappropriate activation of thymocytes during the process of maturation. Antibodies that can inappropriately activate or cause adhesion of thymocytes may heighten thymocyte susceptibility to HIV-1 infection (33) or facilitate intercellular virus transmission. Moreover, thymocyte-specific anti-CD43 antibodies could target thymocytes for destruction by complement-mediated lysis and thus contribute to the severe thymic atrophy in AIDS (34, 35). If the thymus is required for normal replenishment of CD4$^+$ lymphocytes in adult humans as it is in adult mice (36, 37), then thymic dysfunction or destruction would be expected to prevent replenishment of mature CD4$^+$ cells killed by HIV-1.

We observed that 8 of the 30 HIV-1-seropositive subjects tested (27%) had serum anti-CD43 antibodies that could immunoprecipitate CD43 from the SupT1 cells. Yet, 18 of the 30 HIV-1-seropositive subjects (60%) had serum antibodies that stained the SupT1 cell line (see Fig. 1). Because some

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patients may have had anti-CD43 antibodies that could not immunoprecipitate CD43, we tested sera by immunofluorescence for the presence of antibodies that could bind specifically to transfected COS cells that express CD43. By this method, sera from 14 of the 30 HIV-1-seropositive subjects (47%) specifically stained the CD43-expressing COS cells (mean fluorescence intensity of each positive sera ≥ 3 SD above the mean fluorescence intensity obtained using pooled normal human sera). No sera from HIV-1-seronegative homosexual men ($n = 14$) specifically stained the CD43-expressing cells. These data suggest that anti-CD43 autoantibodies are a common feature of HIV-1 infection and provide further evidence that such antibodies are restricted to individuals infected by HIV-1.

The most provocative data suggesting that anti-CD43 autoantibodies may contribute to the immunodeficiency of AIDS come from the study of children with the Wiskott-Aldrich (WA) syndrome, an X chromosome–linked, severe immunodeficiency syndrome. Lymphocytes from children with the WA syndrome express diminished amounts or unstable forms of CD43 (17, 38). The clinical course of the WA syndrome includes progressive T cell depletion, susceptibility to opportunistic and pyogenic infections, inability to produce antibodies against polysaccharide antigens, thrombocytopenia, and severe eczema (39). Several of these clinical features, particularly T cell depletion and susceptibility to opportunistic infections, also characterize HIV-1 infection. If normal CD43 expression is important for T cell development and if anti-CD43 autoantibodies can interfere with this process, then there may be a link between the immunopathogenesis of AIDS and the WA syndrome.

Why are anti-CD43 autoantibodies produced by HIV-1-infected individuals? One possibility is that in noninfected individuals, immunogenic epitopes of CD43 on circulating lymphocytes evade immunologic recognition because they are masked by sialic acid residues. However, in conditions where increased lymphocyte destruction is thought to occur (e.g., HIV-1-induced lymphocyte cytopathicity), autoimmunogenic CD43 epitopes may be exposed. The exposure of such epitopes could result in the induction of autoantibodies, before $CD4^+$ lymphocyte depletion and the corresponding defect in humoral immune responses occur.

Depletion of $CD4^+$ lymphocytes has been noted in many HIV-1-seropositive, healthy individuals (40) when the proportion of virus-infected lymphocytes is estimated to be small (~1:50,000) (41) and plasma viremia is low or undetectable (41, 42). Such observations support the notion that in addition to direct virus cytopathicity, other mechanisms may contribute to $CD4^+$ lymphocyte depletion early in the course of HIV-1 infection. For example, cytolysis of infected $CD4^+$ cells in asymptomatic individuals by CTL combined with defective T cell replenishment could manifest as a selective depletion of the $CD4^+$ lymphocyte pool. Understanding the effects of anti-CD43 autoantibodies on T cell maturation may provide further insight into the immunopathogenesis of $CD4^+$ cell depletion in AIDS.

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