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**Research Article**

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# Deficiency of the Autologous Mixed Lymphocyte Reaction in Patients with Classic Hemophilia Treated with Commercial Factor VIII Concentrate

## Correlation with T Cell Subset Distribution, Antibodies to Lymphadenopathy-Associated or Human T Lymphotropic Virus, and Analysis of the Cellular Basis of the Deficiency

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## Abstract

**14 patients with hemophilia were studied for the distribution of T cell subsets, the presence of antibody to lymphadenopathy-associated or human T lymphotropic virus type III (LAV/HTLV-III), and their responsiveness in autologous mixed lymphocyte reactions. In addition, mitogen and alloantigen responsiveness and Interleukin-2 production were investigated. Seven patients were found to have low Leu 3a/Leu 2a (T4/T8) ratios; eight patients had antibody to LAV/HTLV-III; and an additional patient had acquired immunodeficiency syndrome. Responsiveness to mitogens and alloantigens as well as Interleukin-2 production were comparable with those of healthy individuals. However, patients with low ratio, many of whom had antibodies to LAV/HTLV-III, had a highly deficient autologous mixed lymphocyte reaction. This reduced response of T cells to autologous non-T cells could not be corrected by elimination of Leu 2a/T8 cells, which indicated that there was a preferential loss of the Leu 3a cell subset(s) which responded to autologous non-T cells. Thus, these patients have a deficiency of intercellular communication within their immune system.**

## Introduction

Recently, evidence has accumulated that patients with hemophilia are at high risk to develop abnormalities of the immune system that can culminate in a disease similar to or identical to the acquired immunodeficiency syndrome (AIDS)[1] (1–6). These hemophilic patients—similar to many homosexuals with and without AIDS (7–9)—have a decreased T4/T8 cell ratio ($R_T$) as determined by monoclonal antibodies to these T cell subsets (10–13).

Viral isolation and serologic studies have recently indicated that AIDS is caused by a retrovirus, namely lymphadenopathy-associated virus or human T lymphotropic virus type III (LAV/HTLV-III) (14–17). Other data suggest that hemophilic patients are also infected with this virus, which is probably transmitted via factor VIII concentrate (18–20). Functional analyses of lymphocytes from hemophilic individuals have predominantly revealed reduced responses to conventional mitogens and antigens (3–5, 21). However, more detailed functional analysis of purified T cell subsets of hemophilics have not been performed to our knowledge.

In the present investigation, the immunologic competence of patients with hemophilia has been analyzed by the help of the autologous mixed lymphocyte reaction (AMLR). The AMLR represents a basic in vitro phenomenon of immunoregulation characterized by the proliferative response of T cells to autologous non-T cells without the addition of known antigen, and thus reflects intercellular communication, generating helper, suppressor, and cytotoxic cells (22–30). Purified Leu 3a (T4) but not purified Leu 2a (T8) cells constitute the major responder cell population (31, 32). A reduced AMLR response is characteristic of a variety of diseases with known or presumed immune abnormalities (32–41), and in the majority of these diseases the defect could be shown to reside in the T responder cells (34–37, 39–41). With regard to dissection of the immune response, the AMLR has several advantages: it represents self-recognition and thus intercellular communication (22–24); it does not depend upon presence of known or added antigens or mitogens (25–27); both macrophages (M$\phi$) and B cells can be used as stimulator cells (42, 43); and the overall AMLR response can be studied as well as the response of purified T cell subsets (31, 32).

In the present study we have therefore employed the AMLR as a probe for the analysis of the immune reactivity of patients with hemophilia in order to define more precisely the abnormality present in these patients. Our data lead to the conclusion that an important T cell subset is missing in patients with hemophilia and low $R_T$, and most of them have antibodies to LAV/HTLV-III.

## Methods

*Patients and controls.* 14 patients with hemophilia A whose clinical characteristics are shown in Table I entered the study. One patient suffered from AIDS with repeated opportunistic infections, lymphadenopathy, and marked lymphopenia. The other patients had neither

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1. *Abbreviations used in this paper:* AIDS, acquired immune deficiency syndrome; AMLR, autologous mixed lymphocyte reaction; CM, complete medium; Con A, concanavalin A; IL-2, interleukin-2; LAV/HTLV-III, lymphadenopathy-associated virus or human T lymphotropic virus type III; MHC, major histocompatibility complex; MLR, mixed lymphocyte cultures; M$\phi$, macrophages; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; $R_T$, T4/T8 cell ratio.

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opportunistic infections nor prodromal signs of an acquired immunodeficiency state. The majority of the patients had been intensively treated over many years with commercial Factor VIII concentrates prepared from large plasma pools. 10 healthy age-matched individuals (eight males, two females) served as controls for functional assays; 16 age-matched individuals served as controls for T cell subset determinations.

*Peripheral blood mononuclear cells (PBMC).* PBMC were isolated by gradient centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Upsala, Sweden) and were then subjected either to staining with monoclonal antibodies or to further separation of subpopulations. Adherent cells (M$\phi$), nonadherent non-T cells (B+null cells), and T cells were isolated as described previously (43) using consecutive application of plastic adherence and gradient centrifugation of nonadherent PBMC after incubation with sheep erythrocytes.

*Determination of lymphocyte subset distribution with monoclonal antibodies.* The monoclonal antibodies employed were anti-Leu 4 (all T cells), anti-Leu 3a (helper/inducer cell subset), and anti-Leu 2a (suppressor/cytotoxic cell subset) (44); all were from Becton-Dickinson & Co., Sunnyvale, CA). The Leu 3a T cell population (identical to the T4 population) is restricted to recognition of class II major histocompatibility (MHC) antigens and contains helper/inducer cells as well as class II specific cytotoxic cells; the Leu 2a T cell population (identical to the T8 population) is restricted to recognition of class I MHC antigens and contains suppressor cells and class I restricted cytotoxic T cells (45); moreover, both the Leu 3a/T4 and the Leu 2a/T8 T cell populations can elaborate interleukin-2 (IL-2) upon appropriate stimulation (46).

Incubation was performed using standard procedures (31), and the cells were counterstained with $F(ab')_2$ fluorescein isothiocyanate goat anti-mouse-immunoglobulin. After washing, cells were analyzed with a fluorescence-activated cell sorter (FACS 440; Becton-Dickinson & Co., Sunnyvale, CA) and the percentage of stained cells was determined. In addition, total counts of the individual cell populations and the ratios of Leu 3a/Leu 2a cells ($R_T$) were calculated for all patients.

*Culture medium and culture conditions.* The medium used for cell cultures was RPMI (Grand Island Biological Corp., Frankfurt, Federal Republic of Germany) supplemented with 10% heat-inactivated human type AB serum (Immuno Diagnostica, Vienna, Austria), 0.5% sodium bicarbonate, 10 mM Hepes buffer, 2 mM L-glutamine, 50 U/ml penicillin, and 50 $\mu$g/ml streptomycin (all from Grand Island Biological Corp.). This medium will be referred to as complete medium (CM).

Incubation of all cultures was performed at 37°C in a humidified atmosphere of 5% $CO_2$ in air for the time indicated. 1 $\mu$Ci of [$^3$H]thymidine (Amersham Corp., Oxfordshire, England) was added to the cultures either 20 h (7-d cultures) or 6 h (4-d cultures) before termination. The cultures were harvested onto glassfiber filters using a semiautomatic harvesting system (Skatron-Flow GmbH, Meckenheim, Federal Republic of Germany); filters were then processed for liquid scintillation counting and counted in a liquid scintillation counter (Beckman Instruments Inc., Scientific Instruments Div., Irving, CA). All cultures were performed in triplicates or quadruplicates, and the results were expressed as geometric means of $\Delta$cpm (i.e., mean cpm of test cultures minus mean cpm of control cultures).

*Lymphocyte transformation with mitogens.* $1 \times 10^5$ T cells (supplemented with 10% autologous M$\phi$) were incubated together with mitogens for 4 d in a total volume of 200 $\mu$l of CM in flat-bottom microtiter wells (Falcon Plastics, Oxnard, CA). The mitogens employed were concanavalin A (Con A; Pharmacia Fine Chemicals, Uppsala, Sweden) at concentrations of 20 and 100 $\mu$g/ml, and purified phytohemagglutinin (PHA; Wellcome, England) at concentrations of 6 and 25 $\mu$g/ml.

*Autologous mixed lymphocyte cultures.* AMLR were performed as described previously (31, 43) using $1 \times 10^5$ T cells as responder cells and $1 \times 10^5$ M$\phi$ or (B+null) cells as stimulator cells; stimulator cells had been pretreated with mitomycin C (Sigma Chemical Co., St. Louis, MO). Cultures were performed in round-bottom microtiter plates (Falcon Plastics) in a total volume of 200 $\mu$l of CM for 7 d.

In some experiments, T cells were fractionated into Leu 3a (T4) or Leu 2a (T8) cells by preincubation with monoclonal antibodies OKT 8 or OKT 4 (Ortho Pharmaceutical, Raritan, NJ) plus complement, respectively, and then used as responder cells. This procedure, which led to an enrichment of >90% of the respective T cell subpopulations as judged by fluorescence-activated cell sorter analysis, has been described in detail previously (31, 32). In these experiments, T cells pretreated with complement alone were used as control responder cell population, and the numbers of viable Leu 3a and Leu 2a T cells were adjusted to the number of unfractionated responder T cells.

*Allogeneic mixed lymphocyte cultures (MLR).* MLR were performed using $1 \times 10^5$ responder T cells and $1 \times 10^5$ allogeneic, mitomycin C pretreated M$\phi$, or (B+null) cells as stimulator cells. Conditions for incubation, processing of cultures, and expression of results were as detailed above.

*Production of interleukin-2.* IL-2 production was induced by incubating $1 \times 10^6$ T cells (supplemented with 10% autologous M$\phi$) with 10 $\mu$g/ml of Con A and 10 ng/ml of phorbolmyristic acetate (Sigma Chemical Co.) in a total volume of 1 ml in 24-well plates (Costar, Cambridge, MA) for 2 d. Supernatants were harvested and frozen at −20°C until analysis. Analysis of IL-2 concentrations in supernatants was performed as described previously using an IL-2 dependent cytotoxic T cell line (47). Results were expressed as units per milliliters after comparison with a standard curve with purified IL-2 (46).

*Determination of antibodies to LAV/HTLV-III.* For this purpose, frozen serum samples from the hemophilic patients were shipped to the Centers for Disease Control, Atlanta, GA. Antibodies to the p 25, p 41, and p 18 antigens of LAV were determined by Western blot as described previously (18).

## Results

*T cells and T cell subsets.* Results of analyses of peripheral T cells and T cell subsets by monoclonal antibodies can be seen in Table I. The principal difference between patients and controls was the reduction of the mean $R_T$. This overall reduction was due to the low $R_T$ in 50% of the patients ($R_T$ 0.17–0.94). In patients who had received >200,000 U of Factor VIII concentrate in the past, mean $R_T$ was 0.77±0.58, which was lower ($P < 0.05$) than the mean $R_T$ in patients having been transfused with <200,000 U of concentrate (1.69±0.83). The ratio did not correlate with the period that had passed since the last concentrate transfusion.

*Antibodies to LAV/HTLV-III.* Antibodies to the p 25 and the p 41 and/or the p 18 antigens of LAV were found in eight patients (No.(s) 2, 4, 5, 6, 9, 11, 13, and 14). The absence of antibody in the AIDS patient (No. 1) was in accordance with a previous notion of the lower incidence of anti-LAV/HTLV-III in hemophiliacs with, when compared with those without, AIDS (18).

*AMLR.* When T cells were stimulated with autologous M$\phi$ or (B+null) cells, significant differences were observed between patients and controls (Fig. 1). Using M$\phi$ as stimulator population, a logarithmic mean of 4,677 cpm was observed in patients as compared with 17,783 cpm in healthy controls; 9 of the 14 patients (64%) but only 1 of 9 controls (11%) had proliferative responses < 10,000 cpm ($P < 0.03$); similar results were observed when (B+null) cells were employed as stimulators: 7 of 13 patients (54%) but only 1 of 10 controls (10%) had AMLR responses < 10,000 cpm, and the patients had a significantly lower logarithmic mean AMLR response than the controls (5,370 vs. 50,582 cpm, $P < 0.02$). Thus, hemophilic patients, as a group, had significantly reduced reactivity in the AMLR. It should be noted that the lowest AMLR proliferation of this study was observed in the patient with hemophilia and

*Table I. Clinical Characteristics of 14 Hemophilic Patients Who Entered the Study**

| Patients | | Percentage among PBMC | | | | Total counts/mm³ | | | | Factor VIII concentrate | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | Age | Leu 4 population | Leu 3a population | Leu 2a population | $R_T$‡ | Lymphocyte | Leu 4 population | Leu 3a population | Leu 2a population | Units§ | Last‖ | Remarks |
| 1 | 16 | 60 | 18 | 32 | 0.56 | 232 | 139 | 42 | 74 | 1,500,000 | 3 | AIDS |
| 2 | 15 | 87 | 24 | 58 | 0.42 | 3,276 | 2,850 | 786 | 1,900 | 93,000 | 8 | |
| 3 | 13 | 70 | 33 | 35 | 0.94 | 1,680 | 1,176 | 554 | 588 | 17,000 | 26 | |
| 4 | 27 | 93 | 12 | 71 | 0.17 | 4,794 | 4,458 | 575 | 3,403 | 665,000 | 4 | |
| 5 | 21 | 94 | 22 | 67 | 0.33 | 2,048 | 1,925 | 451 | 1,372 | 415,000 | 18 | |
| 6 | 23 | 70 | 41 | 22 | 1.86 | 1,580 | 1,106 | 648 | 348 | 356,000 | 4 | |
| 7 | 26 | 70 | 38 | 25 | 1.52 | 1,961 | 1,373 | 745 | 490 | 26,000 | 6 | INH |
| 8 | 30 | 75 | 54 | 22 | 2.45 | 1,917 | 1,439 | 1,035 | 422 | 100,000 | 390 | INH |
| 9 | 13 | 85 | 52 | 35 | 1.48 | 1,848 | 1,571 | 961 | 647 | 47,000 | 14 | |
| 10 | 25 | 65 | 44 | 18 | 2.44 | 1,975 | 1,284 | 869 | 356 | 20,000 | 76 | |
| 11 | 33 | 81 | 38 | 45 | 0.85 | 1,218 | 987 | 463 | 548 | 1,330,000 | 1 | INH |
| 12 | 21 | 79 | 49 | 19 | 2.58 | 3,800 | 2,528 | 1,568 | 608 | 166,000 | 21 | |
| 13 | 23 | 60 | 30 | 26 | 1.15 | 3,078 | 1,847 | 923 | 800 | 1,080,000 | 1 | |
| 14 | 15 | 62 | 19 | 40 | 0.47 | 3,234 | 2,005 | 614 | 1,294 | 612,000 | 1 | |
| Means | | 73.3±15.5 | 33.9±13.5 | 36.8±17.5 | 1.23±0.84 | | 1,729±1,045 | 731±352 | 918±864 | | | |

T cells and T cell subsets in healthy individuals ($n$ = 16) were as follows: Leu 4 cells: 73±5% (1,365±263 cells/mm³); Leu 3a cells: 45±6% (840±167 cells/mm³); Leu 2a cells: 22±6% (407±153 cells/mm³); $R_T$: 2.3±0.9. Using $t$ test, the differences between patients and controls were significant for percentages of Leu 3a cells ($P < 0.01$) and Leu 2a cells ($P < 0.01$) as well as for $R_T$ ($P < 0,005$). INH, circulating Factor VIII inhibitor. * In addition, results of low cytometry analyses of PBMC with monoclonal antibodies are shown and the total counts of the respective populations are calculated. ‡ $R_T$, Leu 3a/Leu 2a. § Total number of units of Factor VIII concentrate transfused before this study. ‖ Weeks that elapsed since the last application of Factor VIII concentrate before this study.

AIDS (No. 1); as can be seen from the figures, this was a virtually absent AMLR reactivity, whereas other patients with low $R_T$ still showed significant albeit very low AMLR proliferation.

Next, experiments were performed to find the causes of the AMLR deficiency. First of all, it was observed that the reduction of the mean AMLR response in the patient group was primarily due to low proliferative responses of lymphocytes from patients with low $R_T$. Thus, using M$\phi$ as stimulators, patients with $R_T < 1.0$ had geometric mean cpm of 955 as compared with 22,909 cpm in patients with $R_T > 1.0$; using (B+null) cells as stimulators, geometric mean cpm were 912 and 42,659, respectively ($P < 0.01$). Moreover, there was a significant correlation between AMLR response and $R_T$ (Fig. 2).

In patients with antibodies to LAV/HTLV-III or AIDS, mean responses to M$\phi$ were 2,398 cpm as opposed to 15,849 cpm in the remaining patients ($0.1 > P > 0.05$); using (B+null) cells as stimulators, means of 2,512 and 28,184 cpm, respectively, were observed ($P < 0.05$).

The correlation of low AMLR with low $R_T$ and presence of antibodies to LAV/HTLV-III did not help sufficiently to understand the mechanisms responsible for the low AMLR response; thus, one possibility to explain the observation was the mere numerical shift of the T cell subsets: since Leu 2a/

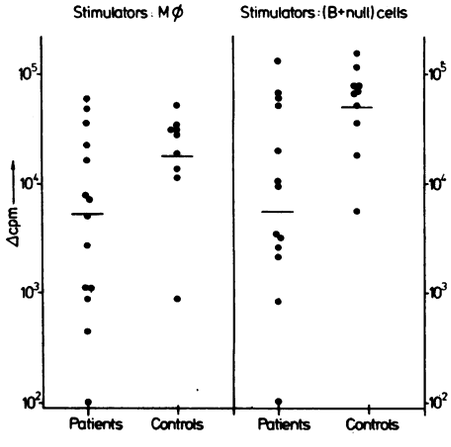


*Figure 1.* Results of AMLR using adherent cells (M$\phi$) or (B+null) cells as stimulators.

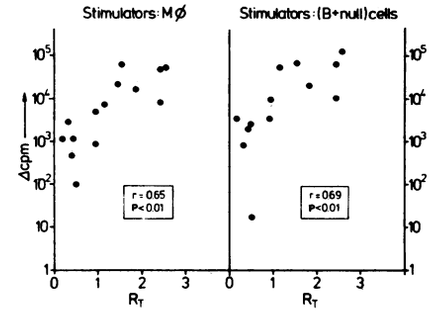


*Figure 2.* The AMLR of patients with hemophilia correlates with the ratio of the major T cell subsets. $R_T$ indicates the Leu 3a/Leu 2a (T4/T8) ratio.

T8 cells, when purified, are nonresponders in the AMLR (31, 48), and since an excess of Leu 2a/T8 cells in mixtures of Leu 2a/T8 and Leu 3a/T4 cells leads to a reduction of AMLR proliferation (32), the observation in the present study could simply be due to the excess of nonresponder cells in patients with low $R_T$. Alternatively, the AMLR-reactive subset of Leu 3a/T4 cells could have been deficient in function or due to physical elimination. To answer this question, experiments were performed in which Leu 2a/T8 cells were eliminated from the T cell pool by monoclonal antibody and complement and then the AMLR was performed. This approach should lead to an increase in AMLR proliferation of patient's lymphocytes if the main problem was an excess of T8 cells (32, 35).

As can be seen from the data shown in Table II, purified Leu 3a/T4 cells from patients with hemophilia and low $R_T$ failed to respond appropriately to non-T cell stimuli, which indicated that in addition to a low $R_T$ these patients had a deficiency of the T cell subpopulation that recognized self-structures on non-T cells.

*MLR, mitogen-induced lymphocyte transformation, and IL-2 production.* To investigate whether the observed reduced AMLR response represented a specific defect or was simply another characteristic of a more global T cell deficiency, lymphocyte transformation induced by allogeneic cells and standard T cell mitogens was investigated. As can be seen from the data presented in Table III, T cells from hemophilic patients, regardless of their $R_T$ or their anti-LAV/HTLV-III antibody status, were competent as responder cells in allogeneic MLR. Also, the stimulatory capacity of patients' non-T cells were well within the normal range.

When T cells (supplemented with 10% autologous M$\phi$) from six of the patients and five of the controls were stimulated with various doses of different mitogens, there was no significant difference between the two populations. Thus, for example, logarithmic mean optimal responses for Con A were 51,286 cpm in patients and 69,183 cpm in controls analyzed in parallel; similarly, no significant differences were observed between the two populations for suboptimal Con A responses or for optimal or suboptimal PHA responses (data not shown). Three of the patients investigated for mitogen reactivity had low $R_T$ (mean Con A response 50,505 cpm) and three had normal $R_T$ (mean Con A response 52,481 cpm). It is of interest that the patient with AIDS (No. 1) had an optimal response to Con A of 32,416 cpm and to PHA of 92,663 cpm; thus, his capacity to proliferate to mitogenic stimuli was not deficient. The mitogenic response of patients with evidence of anti-LAV/HTLV-III or AIDS also did not differ from responses of patients without evidence of anti-LAV/HTLV-III antibodies.

With regard to IL-2 production which was induced by stimulating T cells with Con A and phorbol-myristic acetate, cells from patients and controls released comparable amounts of IL-2 into the supernatants (325±137 U/ml in patients vs. 182±63 in controls, $n$ = 5 and 4, respectively). Again, no association could be seen between IL-2 production and $R_T$ or anti-LAV antibody status.

*Table II. Elimination of T8/Leu 2a Cells Does Not Correct the AMLR Defect in Hemophilic Patients with $R_T$ < 1.0*

| Group | AMLR response of: Unfractionated T cells | AMLR response of: Purified T4 cells* |
|---|---|---|
| Hemophilic, $R_T$ < 1.0 ($n$ = 4) | 1,072 (3.03±0.31)‡ | 871 (2.94±0.72) |
| Hemophilic, $R_T$ > 1.0 ($n$ = 3) | 20,417 (4.31±0.38) | 25,703 (4.41±0.31) |
| Healthy controls ($n$ = 5) | 27,542 (4.44±0.26) | 31,622 (4.50±0.30) |

* T cells pretreated with OKT 8 monoclonal antibody and complement. The data shown have been obtained using M$\phi$ as stimulator cells; similar results were obtained with (B + null) cells as stimulators (data not shown).
‡ Reconversed value of logarithmic mean Δcpm; the numbers in parentheses represent the logarithmic means±SD. The differences between hemophilic patients with $R_T$ < 1.0 and the other two groups were statistically significant at levels of $P$ < 0.02 to $P$ < 0.001 ($t$ test).

*Table III. T Cells from Patients with Hemophilia Respond Well to Stimulation by Allogeneic Non-T Cells*

| Source of T cells | Source of Non-T cells | cpm obtained using (B + null) | cpm obtained using Mϕ |
|---|---|---|---|
| Patient 2 | Control A | 316,286 | 64,349 |
| Patient 3 | Control A | 217,702 | 257,609 |
| Patient 4 | Control B | 198,521 | 209,638 |
| Patient 5 | Control B | 265,648 | 243,910 |
| Patient 6 | Control C | 101,400 | 98,516 |
| Patient 7 | Control C | 74,418 | 116,180 |
| Patient 14 | Control D | 155,497 | 80,215 |
| Control B | Patient 4 | 299,490 | 103,540 |
| Control C | Patient 6 | 135,622 | 63,626 |
| Control D | Patient 14 | 288,900 | 102,706 |

MLR results of pairs of healthy controls (i.e., responder cells from a healthy donor, stimulator cells from an allogeneic healthy donor) performed at the same time in our laboratory were: (B + null) stimulators: 86,823, 41,199, 148,370, 225,101, 337,869, 169,006, and 66,482 cpm. M$\phi$ as stimulators: 32,568, not done, 82,285, 106,169, 151,280, 28,474, and 107,603. Thus, the responsiveness (upper part of the table) as well as the stimulatory capacity (lower part) of the patients were well within normal range. No significant differences were observed between the MLR results from patients and controls by $t$ test. Antibody to LAV/HTLV-III was found in serum of patients 2, 4, 5, 6, and 14.

## Discussion

The present study was directed at defining the cellular basis of the immunologic abnormalities of patients with classic hemophilia. The major defect observed was a severe derangement of intercellular communication in a subgroup of patients with hemophilia, namely those with low Leu 3a/Leu 2a (T4/T8) ratios. This derangement of cell-cell interaction could be recognized by investigation of the AMLR, which represents a basic immunoregulatory phenomenon (32, 33, 49).

It has been recently established that patients with AIDS and many of those predisposed to develop AIDS suffer from an infection with LAV/HTLV-III (14–17). In the present investigation, antibodies to antigens of this virus could also be demonstrated in sera of patients with hemophilia, which indicates that they may have been exposed to LAV/HTLV-III

antigens. These data confirm recent similar evidence (18, 19) and represent a part of a large multicenter study regarding anti-LAV/HTLV-III status in hemophilic individuals.[2] Among the patients analyzed here, 57% had antibodies to LAV/HTLV-III, and one patient had AIDS but had no antibody demonstrable in his serum. (The absence of antibody in this patient is no indication against LAV/HTLV-III infection, since absence of antibody has been observed in a large number of patients with AIDS with or without concomitant hemophilia [18, 50]. Moreover, virus could be isolated from lymphocytes of antibody-negative individuals [51]). Patients with anti-LAV/HTLV-III, in this study, had lower mean $R_T$ values and lower AMLR responses than the remaining patient population.

LAV-HTLV-III has a selective tropism for Leu 3a/T4 (helper/inducer) cells and is cytopathic for those cells (52). It is this cell population that constitutes the responder cell population in the AMLR (31, 32, 48). In hemophilic patients with low $R_T$, but not those with normal $R_T$, responsiveness of purified Leu 3a/T4 cells was severely deficient. This defect was observed when two types of stimulator cells, M$\phi$ or (B+null) cells, were employed. Therefore, the abnormality was not due to a reduced capacity of a subset of non-T cells from hemophiliacs to stimulate the AMLR, but due to a severe deficiency of the responder cell population.

In systemic lupus erythematosus, Hodgkin's Disease, and several other disorders with impaired AMLR responses (34–37, 39–41), a defect of T cell responsiveness in the AMLR has also been observed. However, it was not possible to determine the mechanisms leading to this defect as yet. Moreoever, in some patients, for example in a subgroup of patients with systemic lupus erythematosus and low $R_T$ (53), part of the defect could be due to an excess of the nonresponder Leu 2a/T8 subset in unfractionated T cell populations (35). With regard to hemophilic patients and deficient AMLR, the present study does not lead to similar conclusions. Rather, patients with hemophilia and low $R_T$ appear to have a functional deficiency of the AMLR-responsive Leu 3a/T4 cell subset. In view of the inducer cell tropism of LAV/HTLV-III and the evidence provided elsewhere[2] and in this investigation that hemophiliacs receiving Factor VIII concentrate may be exposed to this virus, it is conceivable that the AMLR-responsive T cell subset has been eliminated by cytopathic effects of the virus. Among all the T cell subpopulations in general, and the Leu 3a/T4 cell subpopulations in particular, the AMLR responsive T cell subset would be the one most prone to such injury, because self-recognition, the main feature of the AMLR, is a continuing event within the immune system, and because the AMLR-reactive T cell pool contains antigen-reactive T cells (54, 55) that become activated in the early phase of every specific immune response.

In additional investigations, mitogen and alloantigen responsiveness was found to be similar in patients and healthy controls; in this respect, our study is at some variance with previous reports which have shown reduced responses in hemophilic individuals (3–5, 21). However, theoretical as well as practical reasons can be discussed in this context: First, the Leu 2a/T8 subset is known to respond well to mitogens and alloantigens in healthy individuals; thus, even for patients with low $R_T$ there is no theoretical reason to be bad responders in these assays; second, PBMC have been used in the quoted studies, whereas purified T cells with a defined accessory cell supplement have been employed in the present investigation, an approach that overcomes potential problems with differences regarding the distribution of the populations of immunocompetent cells between individual patients or study populations. Furthermore, the observation of normal IL-2 production in hemophilic patients irrespective of their $R_T$ indirectly confirms the finding of normal proliferative responses to mitogens. This observation is also not surprising, since T8 cells have been shown to secrete IL-2 in similar amounts as T4 cells (46).

In conclusion, the AMLR (but not a variety of other functional tests employed in this study) has been a useful tool for the analysis of T cell competence in the context of this investigation. Some patients with hemophilia are severely deficient in their AMLR responsiveness, and this deficiency is due to a significant reduction of the specific T cell subsets involved in self-recognition. In patients with AIDS, such as the one studied here, there may be even a complete loss of this T cell population as indicated by the virtual absence of an AMLR response. The Leu 3a/T4 cell subset(s) remaining in the circulation of the patients with low $R_T$ and low AMLR proliferation may predominantly constitute subset(s) not involved in self-recognition; this could be further studied by the help of monoclonal antibodies to differentiation antigens subdividing the major T cell populations. Although the majority of the patients with low AMLR also had antibodies to LAV/HTLV-III, the presence of the antibody does not necessarily indicate immunologic incompetence, since some patients with demonstrable antibody had normal AMLR-proliferation; on the other hand, failure to demonstrate these anti-viral antibodies does not exclude lack of immunocompetence, as could be shown by absent AMLR responsiveness in the patient with concomitant AIDS but negative antibody test and in an additional patient without demonstrable antibody, but very low AMLR. Similar conclusions can be drawn from clinical observations (18).[2] Thus, analysis of the AMLR in patients at risk to develop AIDS may shed more light on the immunologic events associated with the syndrome and the degree of immunologic competence in individual patients.

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