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22. This work was supported by NIH grant AI 18710 to M.S., NSF grant DMR 8404001 to D.C.N., and a Biomedical Research Support (BRS) institutional grant, SO-7-RR05700-15, Medical College of Ohio. We are particularly grateful to C. Pettinga for his assistance in obtaining the support of a grant from the Eli Lilly Company. We are indebted to the Sandoz Company, Basel, for a gift of lysine vasopressin, and to Ferring, A.B., Malmo, Sweden, for a gift of DDAVP. The Glucometer was a gift from the American Diabetes Association Ohio Affiliate, Inc., Toledo. We thank our colleagues R. Raeder, E. H. Freimer, and F. McCullough for help and advice and C. Bedra, L. Kecskes, and R. Taylor for constructing the apparatus. Student assistants were C. Collins, S. Glover, L. Saffran, P. Graham, K. Steenbergh, and M. Sturm. M.S. thanks D. M. Matthews and D. Burston for their hospitality and patience during a month's visit to their laboratory at the Westminster Hospital, London, to learn techniques of studying intestinal absorption. The trip was made possible by a travel award from the Wellcome Trust.

6 December 1985; accepted 14 July 1986

Direct Polyclonal Activation of Human B Lymphocytes by the Acquired Immune Deficiency Syndrome Virus

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When B lymphocytes from normal human peripheral blood were incubated for 1 hour with the retrovirus that causes the acquired immune deficiency syndrome (AIDS), the B cells showed marked proliferation and differentiation. Proliferative responses to the virus peaked on day 4 and appeared to be independent of accessory cells. This finding was repeated with three separate viral isolates, one of which was from a patient from Zaire. The magnitude of the observed responses was comparable to that seen with standard polyclonal B-cell activators. This phenomenon may be at least partially responsible for the polyclonal B-cell activation seen in patients with AIDS.

THE ETIOLOGIC AGENT OF THE ACquired immune deficiency syndrome (AIDS), known as human T-lymphotropic virus type III or lymphadenopathy-associated virus (HTLV-III/LAV), selectively infects and destroys the helper/inducer subpopulation of human T lymphocytes (*1*). This results in a severe crippling of the immune system at the T-cell level. There is good evidence that the T4 molecule is the receptor for the virus, not only on helper/inducer T cells but on a variety of cells and cell lines (*2*). Among the immunologic abnormalities that are thought to be a direct result of infection with the AIDS virus are lymphopenia, which is predominantly due to the quantitative decrease in helper/inducer T lymphocytes, decreased immunologic responses to soluble antigens, and decreased virus-specific cytotoxicity (*3*). In contrast to these depressive effects on the function of T cells, infection with the AIDS virus results in an intense polyclonal activation of B cells. This is evidenced by elevated serum levels of immunoglobulins G and A (IgG and IgA), the presence of circulating immune complexes, and an increased number of peripheral blood lymphocytes spontaneously secreting immunoglobulin (*4*).

Several mechanisms have been proposed to explain the polyclonal B-cell activation seen in patients with AIDS. Among them are the T-cell independent activation and transformation of B lymphocytes by Epstein-Barr virus (EBV) or cytomegalovirus in the absence of the normal immunoregulatory T-cell influences, the production of B-cell activating factors by AIDS virus–infected T cells, and a direct activation of B cells by the AIDS virus. An understanding of the interaction of the AIDS virus with B lymphocytes might provide additional insights into the immunopathogenesis of AIDS and, in particular, might suggest that receptor molecules other than T4 could play a role. In the present study we demonstrate that the AIDS virus can induce B-cell proliferation and immunoglobulin secretion to a degree that is comparable with any of the currently used polyclonal B-cell activators.

For these experiments, human peripheral blood mononuclear cells were obtained from healthy individuals who were seronegative for antibodies to the AIDS virus. They were greatly enriched for B cells by means of Hypaque-Ficoll separation and depletion of monocytes by passage through G-10 columns. Depletion of T cells was then achieved by rosetting with sheep red blood cells that had been treated with 2-aminoethylisothiouronium bromide (*5*). The resulting cell suspensions contained 70 to 85% B cells and less than 5% T cells, as determined by phenotypic and functional analysis with the use of monoclonal antibodies to the CD3 complex; a polyvalent antiserum to surface immunoglobulin; and proliferative responses to phytohemagglutinin (PHA), a pure T-cell mitogen, and whole, Formalin-treated *Staphylococcus aureus* Cowan strain I, a pure B-cell mitogen. While cell separation techniques such as sorting by FACS (fluorescence-activated cell sorting) are capable of providing more highly purified populations of B cells, the large numbers of cells required in our studies made such methods infeasible.

Supernatants containing different isolates of the AIDS virus were obtained by growing the viruses either in peripheral blood mononuclear cells that had been stimulated for 3 days with PHA or in the A3.01 cell line (*6*).

Samples of B cells were incubated for 1 hour with A3.01 supernatant containing AIDS virus strain FB-3, and B-cell proliferation, determined by [^{3}H]thymidine incorporation, was measured daily. As shown in Fig. 1, substantial B-cell proliferation was induced by the FB-3 containing supernatant. This proliferation was noted as early as day 2 and peaked on day 4 in all experiments. In contrast to the B-cell activation and transformation that have been described for EBV (*7*), the B-cell proliferation induced by FB-3 was a transient event and was no longer detectable by day 9 of culture. The magnitude of the response (9,000 to 28,000 cpm) was as great as that seen with any of the known T-cell independent human B-cell mitogens, such as *Staphylococcus aureus* Cowan strain I or EBV (*8*).

We next determined the concentration of virus that would result in the greatest prolif-

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Table 1. Effects of HTLV-III/LAV on B-cell proliferation. Supernatants of HTLV-III/LAV–infected A3.01 cell line were placed over a sucrose gradient and subjected to zonal centrifugation at 80,000*g*. The fractions obtained were screened for the presence of viral proteins by Western blot. Fractions containing viral proteins were pooled (pool 2) as were nonviral fractions (pools 1 and 3). The pooled fractions were incubated with B cells and placed in culture as described in Fig. 1. The B cells were harvested on day 4 and [^{3}H]thymidine incorporation was measured.

Pool	[^{3}H]Thymidine incorporation (cpm)	Western blot
1	893	–
2	12,079	+
3	1,099	–
Control	795	

erative response, and investigated the potential toxic properties of the virus. The magnitude of the proliferative response increased as the concentration of the virus increased, with a plateau occurring at a multiplicity of infection of 0.001 to 0.002 infectious units per B cell (Fig. 2). We were unable to produce higher concentrations of virus than those studied and thus could not determine with certainty whether cytotoxic effects would be seen at a higher multiplicity of infection.

To ensure that this property of B-cell activation was a general property of the AIDS virus and not unique to the particular isolate (FB-3) that was used, we tested several other isolates in the same system. All three isolates tested, including one strain (L-Z84) obtained from a Zairian patient, induced B-cell proliferation (Fig. 3). Furthermore, at day 4 of culture, the proliferative responses induced by all strains of the AIDS virus were greater than the responses induced by EBV. By day 10, B cells incubated with EBV were still proliferating whereas B cells incubated with the AIDS virus exhibited no evidence of viral transformation as determined by the measurement of [^{3}H]thymidine incorporation (Fig. 1) as well as by in situ hybridization with the use of nucleic acid probes (*9*). Supernatants containing viruses grown in peripheral blood mononuclear cells were as capable of inducing B-cell proliferation as were viruses grown in A3.01. Neither supernatants of the noninfected A3.01 cell line nor supernatants obtained from noninfected peripheral blood mononuclear cells stimulated with PHA induced B-cell proliferation in this manner.

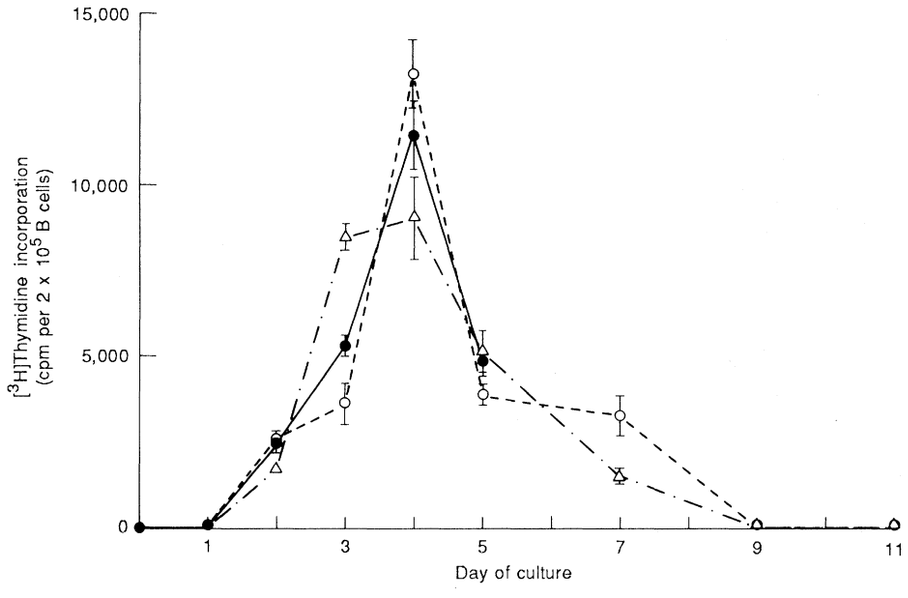

Fig. 1. Kinetics of HTLV-III/LAV–induced B-cell proliferation. Virus-containing supernatants were derived from the HTLV-III/LAV–infected A3.01 cell line. The stock supernatant contained virus at a concentration of 10^4 infectious units per milliliter as defined in Fig. 2. B cells were prepared as described in the text. The B cells (5×10^6) were centrifuged and the pellet was resuspended in 1 ml of virus-containing supernatant and incubated for 1 hour at 37°C. The cells were then washed twice with RPMI 1640 and resuspended in RPMI 1640 containing 10% fetal calf serum (FCS) at 2×10^6 cells per milliliter. Portions (0.1 ml) were placed into round-bottom microtiter dishes already containing 0.1 ml of RPMI 1640 with 10% FCS in each well. The cultures were incubated at 37°C at 7% CO_2. On sequential days, the cultures were incubated for 4 hours with 2 μCi of [^{3}H]thymidine and harvested onto glass fiber filters. Radioactivity was measured in a liquid scintillation counter and expressed as counts per minute per 2×10^5 B cells. The figure depicts three of six such experiments. Each point shows the average (± SEM) for counts from six wells. Background counts of B cells not exposed to virus (range 50 to 1000 cpm) are subtracted from the data.

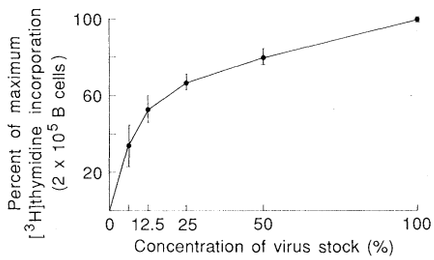

Fig. 2. Effect of concentration of HTLV-III/LAV on B-cell proliferation. Various dilutions of HTLV-III/LAV–infected A3.01 cell line supernatants were incubated with 5×10^6 B cells as described in Fig. 1. The B cells were harvested on day 4. The ordinate represents the percent of the maximum observed [^{3}H]thymidine incorporation for each of the concentrations of virus stock. The undiluted (100%) virus stock represents 10^4 infectious units per milliliter as determined by the reciprocal of the highest dilution that could still induce reverse transcriptase activity and cytopathic effect in a susceptible cell line. Data presented are means ± SEM for four experiments.

To investigate further whether the B-cell activation observed was due to a direct interaction between the B cell and the AIDS virus or was mediated by virus-induced cell products, we performed experiments with supernatants fractionated in the following manner. Samples of A3.01 supernatants containing AIDS virus were pooled, placed over a sucrose gradient, and subjected to zonal centrifugation at 80,000*g*. The fractions obtained were screened by Western blotting for the presence of viral proteins. Fractions containing viral proteins were pooled as were nonviral fractions above and below the peak of viral proteins. These pools were then incubated with B cells for 1 hour and placed in culture. At day 4 of culture we observed significant induction of B-cell proliferation only in those pools that contained the AIDS virus (Table 1). This strongly suggests that the B-cell activation described here is due to a direct interaction between the virus and B cells.

The standard sequence of events in B-cell maturation involves discrete steps of activation, proliferation, and differentiation (*10*). Having demonstrated that the AIDS virus can induce marked B-cell proliferation, we next studied the effect of the virus on immunoglobulin secretion by B lymphocytes. In cultures incubated with virus for only 1 hour, immunoglobulin secretion was substantial at the end of 10 days. This secretion was approximately 50% of that induced by pokeweed, a T- and B-cell mitogen that is T cell–dependent (Table 2), and was greater in magnitude than that induced by any other available T cell–independent B-cell activator (*11*).

Thus, our data show that a variety of isolates of the AIDS virus can induce the

proliferation and differentiation of normal human peripheral blood B lymphocytes. The effect appears to be T cell–independent and is comparable in magnitude to the response that can be induced with the most potent polyclonal B-cell activators currently used in human immunology.

The data suggest that the B-cell activation is due to a direct interaction between the AIDS virus and the B lymphocyte. The fact that the responses occur after a short incubation time, can be seen with virus propagated in either peripheral blood mononuclear cells or the A3.01 cell line, occur with highly concentrated bands of virus obtained with zonal centrifugation, and have none of the kinetic characteristics of the responses induced by the known T cell–derived B-cell activating factors suggests strongly that they are due to a direct interaction between B cells and the virus.

It is difficult to say what role this mechanism plays in the polyclonal B-cell activation that occurs in patients with AIDS. The magnitude of the responses suggests that this type of activation may be the major mechanism responsible for the hypergammaglobulinemia in AIDS patients. However, EBV can be isolated from virtually all AIDS patients (*12*), and B-cell lines transformed with this virus are quite easily obtained from the peripheral blood of many of these patients. Thus, both viruses may contribute to the polyclonal B-cell activation that is characteristic of AIDS. Nevertheless, this new observation may explain the anecdotal reports of hypergammaglobulinemia in pediatric patients with AIDS who do not have evidence of EBV infection (*13*).

Previous reports of the interaction of the AIDS virus with B lymphocytes have been restricted to B lymphocytes that have been infected and transformed with EBV (*14*). However, in the studies described here, B-cell activation was independent of EBV infection as evidenced by the fact that in most of the experiments the healthy lymphocyte donors were seronegative for EBV.

Although the helper/inducer T cell is believed to be the main target of the AIDS virus, a variety of other cells and cell lines are capable of being infected. These include macrophages and, as mentioned above, EBV-positive B lymphocytes (*2, 15*). In all instances, however, these cell lines, in contrast to peripheral blood B cells, have been noted to have the T4 molecule on their surface (*2*). The current observation of B-cell activation by the AIDS virus in the absence of EBV infection suggests that there may be alternative receptor sites for the virus.

Table 2. HTLV-III/LAV–induced B cell immunoglobulin production. B cells were incubated for 1 hour with various dilutions of HTLV-III/LAV isolate FB-3 as described in Fig. 1. Cultures were incubated at 37°C for 10 days and supernatants were then assayed for immunoglobulin secretion by enzyme-linked immunosorbent assay. Data are expressed as nanograms of IgG or IgM per milliliter of culture (± SEM for n = six cultures). The B-cell control represents B cells that were not exposed to virus. The positive control consists of unexposed B cells cultured with irradiated autologous T cells and pokeweed mitogen (PWM).

Culture	Experiment 1		Experiment 2	
	IgG	IgM	IgG	IgM
B cells alone	500 ± 150	0 ± 0	1,300 ± 100	0 ± 0
B cells + irradiated T cells + PWM*	4,600 ± 300	15,200 ± 2,950	13,700 ± 1,400	25,600 ± 2,450
B cells + FB-3 (1:1)	2,750 ± 200	4,450 ± 500	6,200 ± 400	13,700 ± 2,050
B cells + FB-3 (1:2)	2,000 ± 100	2,100 ± 100	3,950 ± 500	10,900 ± 1,450
B cells + FB-3 (1:4)	1,500 ± 50	1,800 ± 200	1,800 ± 150	6,550 ± 1,950
B cells + FB-3 (1:8)	200 ± 50	600 ± 100	3,500 ± 700	2,100 ± 300
B cells + FB-3 (1:16)	350 ± 100	550 ± 200	5,000 ± 1,100	500 ± 50

*The irradiated T cells received 1000 R before being placed in culture.

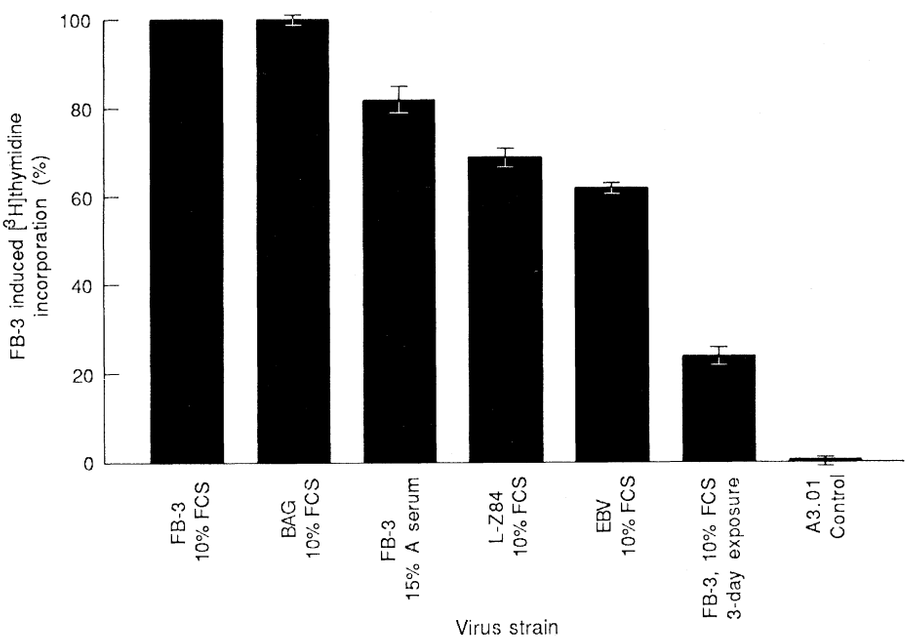

Fig. 3. Comparison of the ability of different HTLV-III/LAV isolates and EBV to induce B-cell proliferation. HTLV-III/LAV isolates FB-3 and BAG were propagated in A3.01 while L-Z84 (obtained from a Zairian patient) was propagated in PHA-stimulated peripheral blood mononuclear cells. EBV-containing supernatants were obtained from an EBV-infected marmoset cell line (B95-8). Undiluted virus-containing supernatants from the infected cell lines were incubated with B cells as described in Fig. 1. Cultures stimulated with FB-3 were also performed after 3 days of exposure to FB-3 or in media containing 15% human A serum rather than FCS. Undiluted noninfected A3.01 cell line supernatant was used as a control. All cultures were exposed to 2 μCi of [^{3}H]thymidine and harvested on day 4 of culture. Results show the means (± SEM) for six separate cultures.

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6 June 1986; accepted 29 July 1986