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Research Article

Human immunodeficiency virus (HIV) infection is associated with a profound impairment of T cell function. Hence, enhancement of T cell reactivity to viral and bacterial antigens is important in the treatment of patients with AIDS. To develop tools for amplifying T cell reactivity, we have immunized mice with human helper T cell clones and selected monoclonal antibodies (MAbs) that enhance in vitro blastogenic responses. MAb NDA5, which recognizes the leukocyte common antigen CD45, amplifies human T cell responses to mitogens and soluble antigens including HIV-1 glycoprotein (gp)-120 and peptides derived from the HIV-1 gp-120 sequence. In the presence of MAb NDA5, peripheral blood mononuclear cells (PBMC) from healthy, HIV-1-seronegative individual displayed augmented blastogenic responses to HIV-1 gp-120 and to HIV-1 gp-120 synthetic peptides. In vitro memory responses to various vaccines and to alloantigens were also enhanced in cultures with MAb. Similarly, the response of PBMC from AIDS patients to pokeweed mitogen, HIV-1 gp-120, and tetanus toxoid was enhanced with MAb NDA5. The finding that the in vitro immune response of patients with AIDS can be amplified with MAb NDA5, suggests that the in vivo immune response of immunodeficient individuals can also be enhanced.

Amplification of T Cell Blastogenic Responses in Healthy Individuals and Patients with Acquired Immunodeficiency Syndrome

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Abstract

Human immunodeficiency virus (HIV) infection is associated with a profound impairment of T cell function. Hence, enhancement of T cell reactivity to viral and bacterial antigens is important in the treatment of patients with AIDS. To develop tools for amplifying T cell reactivity, we have immunized mice with human helper T cell clones and selected monoclonal antibodies (MAbs) that enhance in vitro blastogenic responses. MAb NDA_5, which recognizes the leukocyte common antigen CD45, amplifies human T cell responses to mitogens and soluble antigens including HIV-1 glycoprotein (gp)-120 and peptides derived from the HIV-1 gp-120 sequence. In the presence of MAb NDA_5, peripheral blood mononuclear cells (PBMC) from healthy, HIV-1-seronegative individuals displayed augmented blastogenic responses to HIV-1 gp-120 and to HIV-1 gp-120 synthetic peptides. In vitro memory responses to various vaccines and to alloantigens were also enhanced in cultures with MAb. Similarly, the response of PBMC from AIDS patients to pokeweed mitogen, HIV-1 gp-120, and tetanus toxoid was enhanced with MAb NDA_5. The finding that the in vitro immune response of patients with AIDS can be amplified with MAb NDA_5, suggests that the in vivo immune response of immunodeficient individuals can also be enhanced. (*J. Clin. Invest.* 1990. 85:746–756.) adjuvants • human immunodeficiency virus glycoprotein-120 • leukocyte common antigen CD45

Introduction

The acquired immunodeficiency syndrome (AIDS) is characterized immunologically by depletion and functional impairment of the leukocyte common antigen (LCA)[1] CD4-positive T lymphocyte subset. CD4-positive T lymphocytes are critical to the induction of cell-mediated immunity and control of viral infection. The induction of protective immunity in AIDS patients may depend on restoring T cell immunocompetence. Therefore, a particularly attractive approach to anti-human immunodeficiency virus (HIV) therapy may reside in the enhancement of cellular immune responses. To develop tools for amplifying T cell reactivity, we have immunized mice with human helper T cell clones and selected monoclonal antibodies (MAbs) that enhance in vitro blastogenic responses. In this report we describe the amplification effects displayed by a new MAb, which we named MAb NDA_5, on human T cell responses to HIV-1 glycoprotein (gp)-120, peptides derived from HIV-1 gp-120, and to other soluble antigens.

A series of lymphoid differentiation antigens were recently shown to serve as signal transducers that control T cell proliferation (1–6). These differentiation antigens and some of the biologically active MAbs that recognize them offer routes whereby enhancement of cell-mediated immune responses can be attained. Some of these antigens, such as CD3 (gp50) and CD28 (gp44), are T lineage-specific (7–10), whereas others, including CD11b (LFA-1α), CD18 (LFA-1β), CD43 (gp-95), CD44 (gp-65–85), CD45 (LCA or T200), and CD45R (restricted LCA or T220), are shared by most cells of hematopoietic origin (1–6, 11–17). In solution, MAbs to CD2, CD3, and CD8 inhibit T cell proliferation (1, 2), whereas when immobilized to a solid phase, they initiate T cell proliferation (14). Antibodies against CD28, CD43, and CD44 in soluble form have been shown to enhance T cell proliferative responses to recall antigens, alloantigens, and plant mitogens by delivering early progression signals (3, 4, 9–14). The specific function of these cell membrane glycoproteins and the mechanism underlying the amplification effect are not clearly understood. The LCA, representing a family of glycoproteins that are major components at the surface of all lymphoid and myeloid cells, is defined by the CD 45/45R cluster of MAbs. The various CD45 antibodies have been categorized as MAbs which (*a*) inhibit proliferation of T cells but do not induce aggregation, (*b*) inhibit proliferation and enhance aggregation, (*c*) do not affect proliferation, but enhance aggregation, and (*d*) enhance both proliferation and aggregation (3, 15). Few MAbs belonging to the latter category have been described so far. Their amplification effects have been documented in a system in which T cells were triggered to proliferate by the use of anti-CD3 MAbs and their proliferation was shown to be IL-2 dependent (12, 17). We now describe a MAb, NDA_5, which recognizes the LCA CD45, induces heterotypic and homotypic aggregation of lymphoid cells, and enhances T cell blastogenic responses independent of IL-2. In this report we document the capacity of MAb NDA_5 to amplify T cell blastogenic responses to a variety of mitogens and antigens, including HIV-1 gp-120, and HIV-1 gp-120–derived peptides. Our results demonstrate that a MAb directed against the LCA can augment the in vitro reactivity of PBMC from healthy, HIV-1–seronegative, and from HIV-1–infected individuals.

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1. *Abbreviations used in this paper:* gp, glycoprotein; LBCL, lymphoblastoid cell line; LCA, leukocyte common antigen; PPD, purified protein derivative; TdR, thymidine. TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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Methods

Generation of murine MAb NDA_5. This antibody was generated, as previously described (18), by immunizing a BALB/c mouse with a human alloreactive T cell clone exhibiting helper function. Briefly, human T cells primed in mixed lymphocyte cultures against HLA-D half-identical stimulating cells were cloned by limiting dilutions. Clones were expanded in medium containing IL-2 and irradiated PBL from the original stimulator. The functional characteristics of the clones were determined as previously described (18). Splenocytes, from the hyperimmunized mouse, were fused with NS-1 plasmocytoma cells using the method of Kohler and Milstein (19). Hybridoma supernatants were screened for antibody activity by immunofluorescence cytofluorometry using lymphoid and nonlymphoid cell lines as targets as previously described (20–22). The NDA_5 hybridoma was selected for cloning and further investigation because it reacted with cells of hematopoietic origin but not with carcinoma cell lines of different tissue origin. Its capacity to stimulate T cell reactivity to plant mitogens constituted an additional criterion for selection. This antibody is of the IgM class as determined by Ouchterlony immunodiffusion and was purified from supernatants on a Sephacryl-S-300 column (Pharmacia, Uppsala, Sweden) which was washed with chloroform in endotoxin-free water. As an isotype control, murine IgM produced by MOPC 104E (obtained from Sigma Chemical Co., St. Louis, MO) was used. The amount of murine Ig in hybridoma supernatants was quantitated by ELISA as previously described (20). These MAbs were used as diluted and filtered culture supernatants or as purified Ig. The $F(ab')_2$ fragment of MAb NDA_5 was prepared by tryptic digestion as previously described (23) with the following modifications: 300 μg of MAb NDA_5 in 1 ml of 10 mM Tris, pH 8.0, 150 mM NaCl, and 50 mM $CaCl_2$ was incubated with 1.4 U of TPCK-Trypsin immobilized to agarose (Pierce Chemical Co., Rockford, IL) for 24 h at 24°C. The supernatant was removed and applied to a high-performance gel filtration column (250 mm × 9 mm, S-250, Bio-Rad Laboratories, Richmond, CA) and eluted in a PBS mobile phase at 1 ml/min flow rate. Fractions were collected and analyzed by nonreducing SDS-PAGE. Fractions containing material with apparent molecular mass ranging from < 220 kD to > 150 kD were pooled and tested for their ability to block the binding of FITC MAb NDA_5. The partially purified tryptic digest of MAb NDA_5 blocked the binding of FITC MAb NDA_5 as demonstrated by immunofluorescence.

Flow cytometric analysis and MAbs. Cells were suspended in PBS containing FCS (2.5%), NaN3 (0.1%), and MAb at an appropriate concentration and incubated for 30 min on ice. For indirect immunofluorescence staining, cells were incubated with FITC-conjugated goat $F(Ab')_2$ fragment anti-mouse IgM or IgG (Tago, Inc., Burlingame, CA). Cell surface receptors for the Fc portion of immunoglobulin were blocked using 1% normal human sera.

Flow cytometric analysis was performed on a fluorescence-activated cell sorter (FACStar, Becton, Dickinson & Co., Sunnyvale, CA), equipped with an ion laser (Innova 70, Coherent, Inc., Palo Alto, CA). Debris and dead cells were excluded from the analysis using propidium iodide staining (Sigma Chemical Co.). FITC emission signals were collected using an appropriate filter at 525 nm and amplified logarithmically. In all cases 20,000 events per sample were collected in list mode fashion, stored, and analyzed by a Consort 30 system (Becton, Dickinson & Co.). The threshold of positivity for fluorescence was arbitrarily established on the basis of the negative-control sample of cells incubated without antibody or with isotype-matched MAbs of irrelevant specificity. One-color analysis data are depicted on histograms in which the number of cells appeared in the *y*-axis and the intensity of fluorescence appeared on the *x*-axis. MAb to CD25 were FITC-conjugated and were obtained from Coulter, Inc., Hialeah, FL. Other antibodies used include three different anti-CD45 MAbs. T191, which precipitates 180-, 190-, 200-, and 220-kD bands of CD45, and 19.8.2B9, which is reactive only with the 220-kD isoform, were generously provided by Dr. R. Mittler, Bristol-Myers Co., New York. The third anti-CD45 MAb, named anti-HeL1, reacts also with the 180–220-kD isoforms and was obtained from Becton, Dickinson & Co. (Mountain View, CA).

Preparation of lymphocyte suspensions. PBMC were obtained by Ficoll-Histopaque gradient centrifugation and suspended in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 1 mM nonessential amino acids, 1 mM sodium pyruvate, and 50 μg/ml gentamicin (Gibco Laboratories, Grand Island, NY). Purified T and B cell suspensions were separated from fresh PBMC by first depleting the cells of monocytes by plastic adherence and then rosetting the T cell fraction with neuraminidase-treated sheep red blood cells. Further purification of the T and B cell population was accomplished using Lympho-kwik T and Lympho-kwik B as described by the manufacturer (One Lambda, Inc., Los Angeles, CA).

Determination of cell aggregation. T cells purified from fresh peripheral blood were stained with 5-(and 6) carboxifluoresceine diacetate (Molecular Probes Inc., Eugene, OR) and suspended in complete culture medium at a concentration of 3×10^6/ml; B cells (3×10^6/ml) from the same individual were suspended in medium but left unstained. 2 μl of T or of B cells or 1 μl from each suspension was dispersed in 60-well-Terasaki trays containing 5 μl of mineral oil. 2 μl of MAb NDA_5, $F(Ab')_2$ fragment of MAb NDA_5, or MOPC-104E at a concentration of 1 and 0.5 μg/ml were added to individual wells. All reactions were done in duplicates. Replicate trays were incubated for 18 h at 4°C, 25°C, or 37°C. The size and number of aggregates of fluoresceinated (T cells) and nonfluoresceinated (B) lymphocytes were scored on a tissue-typing fluorescence microscope (E. Leitz, Inc., Rockleigh, NJ) at a magnification of ×150.

Blastogenesis assays. PBMC were plated in 96-well, round-bottom trays (Linbro Chemical Co., New Haven, CT) at a concentration of 1×10^5 in 0.2 ml. PWM (Gibco Laboratories) was added at a final dilution of 1:400 per 10^5 cells. Tetanus toxoid was obtained from the Massachusetts Public Health Biological Laboratory and used at a final dilution of 1:400 in complete culture medium. Purified protein derivative (PPD) of *Mycobacterium tuberculosis* was obtained from Connaught Laboratory (Ontario, Canada) and used at a concentration of 3.5 μg/ml of medium. Hepatitis B vaccine MSD, obtained from Merck, Sharp & Dohme (West Point, PA) was dialyzed against PBS and used at a 1:200 dilution of the original volume. HIV-1 gp-120 was purified as previously described and used at a concentration of 5 μg/ml (24). Synthetic peptides corresponding to segments of HIV-1 gp-120 were kindly supplied by Dr. R. Neurath from the New York Blood Center. Experiments in which the blastogenic response to these peptides and other antigens were compared, were performed using as antigen-presenting cells autologous monocytes pulsed with antigen as described (25). Mixed lymphocyte cultures were performed using equal numbers (1×10^6) of responding and stimulating (2,000 rads) irradiated PBMC from HLA D/DR different blood donors (18).

Activation of purified T cells was induced by culturing T cells (4×10^5) for 3 d with accessory cells (6×10^4) in triplicate wells of 96-well-round bottom trays (Linbro Chemical Co.) in the presence of 25 ng/ml of anti-CD3 MAb (Ortho Diagnostic Systems, Raritan, NJ) or in the presence of 2.0 μg/ml of PHA (Burroughs Wellcome Co., Research Triangle Park, NC). Parallel T cell cultures without accessory cells were stimulated with phorbol myristate acetate (PMA) (0.5 ng/ml) from Sigma Chemical Co. alone or in combination with ionomycin (200 ng/ml) from Calbiochem-Behring Corp. (San Diego, CA). Cultures were labeled with [^{3}H]thymidine (TdR) over the last 18 h of incubation, harvested, and counted in a liquid scintillation counter (1205 Betaplate, LKB Instruments, Inc., Gaithersburg, MD). The mean and standard deviation of counts per minute in triplicate reactions were calculated.

Results

Cell surface expression of NDA_5. The antigen detected by MAb NDA_5 is expressed by granulocytes and by T, B, and monocytic cells isolated from fresh peripheral blood, spleen, and ton-

sils. Similarly, Epstein-Barr virus (EBV)-transformed lymphoblastoid B cells lines, alloreactive T cell clones, Jurkat, MOLT-4, and CEM leukemic T cell lines, human K562 chronic myelogenous leukemic, and gibbon lymphoma MLA-144 express NDA_5. Human erythrocytes, platelets, and cell lines derived from neuroglioma, prostatic and breast carcinoma, and lymphocytes from baboon peripheral blood and spleen did not react with MAb NDA_5, as determined by immunocytofluorometry. Although this antigen is constitutively expressed by resting PBMC, monitoring of the level of expression of NDA_5 on cells stimulated with PWM or with PHA showed a decrease in the percentage of reactive cells and fluorescence intensity at the peak of the blastogenic response on day 6 or day 2, respectively (Fig. 1).

NDA_5 can be distinguished from other antigens shared by various cells of hematopoietic origin such as CD29, CD38, CD43, CD18, and CD11A, yet its tissue distribution and level of expression appears to be similar to that of CD45 (26). Repeated attempts to analyze the molecule recognized by MAb NDA_5 by immunoprecipitation of cell membrane lysates labeled with ^{125}I or labeled metabolically with [^{35}S]methionine were inconclusive probably due to the IgM nature of the antibody. Thus, to establish whether MAb NDA_5 does indeed react with CD45, its immunoreactivity with transfected mouse cell lines that express different isoforms of CD45 was tested (27). MAb NDA_5 reacted in a pattern that suggests that it recognizes an epitope expressed on each CD45 isoform (Schlossman, S., personal communication).

Cell aggregation studies. MAb NDA_5, or its $F(ab')_2$ fragment, induced the aggregation of T cells, B cells, and of mixtures of T and B lymphocytes in plates incubated for 18 h at 25°C or 37°C. No aggregates were found within the first 2 h of incubation with MAb NDA_5. The aggregation triggering effect of MAb NDA_5 is dose dependent since both the size and the number of cell aggregates were higher at 1 μg/ml than at 0.5 μg/ml. This effect was inhibited at 4°C and in the presence of sodium azide, indicating that it is dependent on cell metabolism. Homotypic cell aggregates resembled rosettes which under the optimal testing conditions (incubation at 37°C with 1 μg MAb NDA_5/ml) contained 8–20 T or B lymphocytes. The

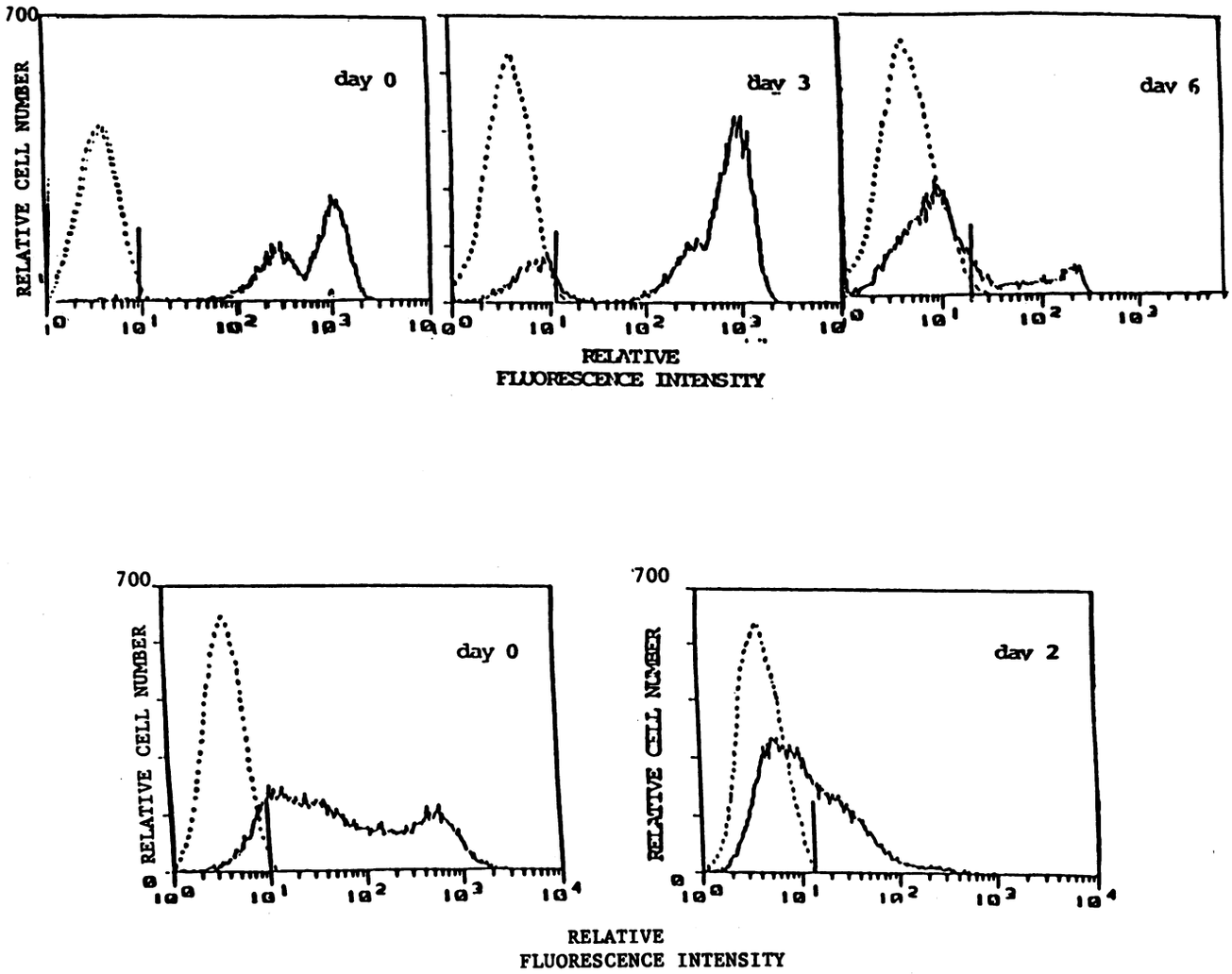

Figure 1. Expression of NDA_5 on PBMC stimulated with mitogens. (*Upper panel*) PWM-stimulated PBMC. (*Lower panel*) PHA-stimulated PBMC. Cells were removed from cultures at indicated times, labeled with FITC-MAb NDA_5, and subjected to FACS analysis.

size of the aggregates was rather similar when T and B cells were mixed together. In such mixtures, most heterotypic rosettes comprised 10–15 T cells and 5–10 B cells. However, these wells also showed a few small aggregates (5–10 cells) with only T or with only B lymphocytes. No aggregates were found under any testing conditions in control reactions containing MAb MOPC-104E or medium only.

Effect of MAb NDA_5 on T cell responses to mitogens. MAb NDA_5 was added, at concentrations ranging from 0.125 to 10 μg/ml, to PBMC which were stimulated with PWM. Control cultures were tested in parallel without MAb or in the presence of MAb MOPC 104E. The magnitude of the blastogenic response was measured after 3 and 5 d of incubation. Fig. 2 shows a side-by-side comparison of results obtained with fresh PBMC from a healthy individual and from a patient with AIDS and Kaposi's sarcoma. MAb NDA_5 did not stimulate lymphocyte blastogenesis in cultures without PWM. MAb NDA_5 enhances and accelerates blastogenic responses to PWM of PBMC from the normal individual. In the absence of MAb NDA_5 the response reaches a peak after 5–6 d and declines progressively thereafter. In the presence of MAb NDA_5, the day 3 response was of the same order of magnitude as the response occurring only 2 d later in cultures without MAb NDA_5. The magnitude of the day 3 and 5 responses were 200% and 50% higher, respectively, in cultures with MAb NDA_5 compared with cultures without MAb or with MAb MOPC 104E. The enhancing effect of MAb NDA_5 was already evident at 0.125 μg/ml, yet it increased in a dose-related manner, reaching the plateau between 1 and 10 μg/ml.

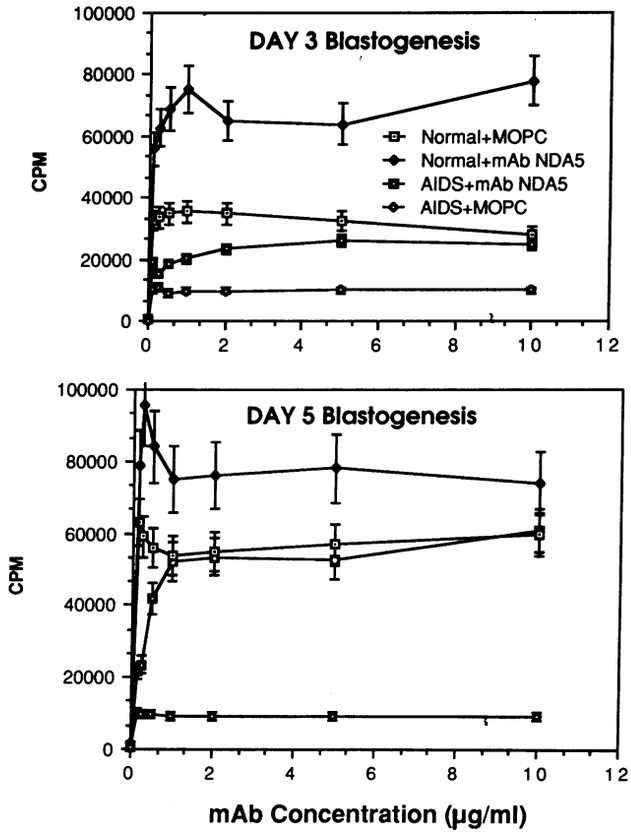

Figure 2. Effect of MAb NDA_5 on the PWM response of a healthy individual and of a patient with AIDS. (*Upper panel*) Day 3 blastogenesis. (*Lower panel*) Day 5 blastogenesis. PBMC were stimulated with PWM in the presence of MAb NDA_5 or control MAb MOPC. [^{3}H]TdR was added over the last 18 h of incubation. *y*-axis, mean cpm of triplicate reactions; *x*-axis, concentration of mAb. The standard deviation of the counts per minute is given by the error bars at each point.

In cultures without MAb NDA_5, both the day 3 and 5 responses exhibited by fresh PBMC from an AIDS patient were much lower than the response of the healthy control. However, MAb NDA_5 at the concentration of 10 μg/ml induced a fourfold augmentation of the day 5 response. Mean counts per minute in such cultures (60,862±3,220) were similar to those observed in parallel cultures containing PBMC from the healthy control, stimulated with PWM without MAb NDA_5 (58,991±4,530). Similar results were obtained in eight other side-by-side comparisons of healthy individuals and patients with AIDS. The potentiating effect of MAb NDA_5 on the reactivity of PBMC from AIDS patients and healthy individuals to PWM was also found in experiments in which cryopreserved cells from patients and controls were used (Table I). Although the overall level of day 3 reactivity of cryopreserved lymphocytes was lower than that observed with fresh lymphocytes, it was still evident that the response of each of the seven patients and six controls was strongly augmented in the presence of MAb NDA_5. Again, in the presence of MAb NDA_5, the level of reactivity of lymphocytes from at least some patients, was > 10,000 cpm, which was similar to that exhibited by cryopreserved lymphocytes from healthy individuals in cultures without MAb NDA_5. The CD3, CD4, and CD8 phenotypes of the T lymphocytes from the AIDS patients included in this study are presented in Table I and show the characteristic inversion of the CD4/CD8 ratio associated with advanced disease. To determine whether the enhancing effect of MAb NDA_5 is due to its being an IgM, we compared the activity of MAb NDA_5 with that of its $F(ab')_2$ fraction in a 3-d PWM blastogenesis assay; two other anti-CD45 MAbs of IgG isotype HEL-1, T191, and its corresponding $F(ab')_2$ were added to parallel, control cultures.

Table I. Potentiating Effect of MAb NDA_5 on the Reactivity of Cryopreserved PBMC to PWM in Healthy Individuals and AIDS Patients with Kaposi's Sarcoma

[^{3}H]TdR incorporation				% T cells in AIDS patients expressing		
Healthy individuals		AIDS patients				
No MAb	MAb NDA_5	No MAb	MAb NDA_5	CD3	CD4	CD8
cpm × 10³						
13.7±1.2	42.2±2.2	0.9±0.1	2.0±0.2	61	19	42
23.7±2.3	60.4±2.5	1.2±0.2	1.5±1.0	46	15	25
12.9±1.2	48.5±1.6	1.8±0.2	6.2±0.5	61	19	42
15.4±1.5	48.3±1.8	2.1±0.1	10.0±0.7	59	24	42
11.8±1.2	37.3±1.3	3.8±0.1	11.5±0.8	62	16	42
16.5±1.6	54.8±2.2	5.6±0.7	13.0±1.4	63	23	36

The PWM response in the presence of MAb NDA5 differed significantly ($P = 0.05$) from the response in the absence of MAb NDA5 in both normal individuals and AIDS patients by the Mann-Whitney U test. The PWM response of AIDS patients in the presence of MAb NDA_5 was not significantly different from the response of normal individuals in the absence of MAb NDA_5 by the same statistical test.

MAb NDA_5 (0.250–1.00 μg/ml) in its native form elicited a strong amplification of the response to PWM. The $F(ab')_2$ fragment of MAb NDA_5 at equivalent concentrations was also stimulatory, although slightly less efficient than the native pentamer. The other anti-CD45 MAbs were slightly inhibitory at the concentrations tested (Table II). Repeat experiments yielded the same results. Thus, the $F(ab')_2$ fragment of MAb NDA_5 has a similar biological activity as its parent IgM, indicating that the enhancing effect of this antibody is not isotype dependent.

To establish whether MAb NDA_5 acts on the cells at the time of activation or after they have received the proliferation signal, the MAbs were added to PBMC together with PWM (time 0), after 4 h, and after 24 h. Quantitation of the blastogenic response on day 3 showed that the amplification effect occurred only in cultures to which MAb NDA_5 was added at initiation (Table III). A similar amplification effect was also observed when PBMC were incubated with MAb NDA_5 (5 μg/ml) for 1 h at room temperature, washed four times, and then stimulated with PWM. The day 3 response was significantly augmented compared with cultures incubated in MOPC 104E (Table III). These results suggest that MAb NDA_5 enhances T cell activation and that its effect occurs before IL-2 receptors are being expressed.

It was not clear, however, from these experiments whether the amplification effect is mediated by T cells, since B cells also participate in the PWM response. Furthermore, it was of interest to determine whether MAb NDA_5 amplifies the direct (CD3-Ti–mediated) and/or alternative pathway of T cell activation. The direct or "antigen pathway" of activation, is initiated by the binding of the appropriate ligand to the CD3-TcR complex, while the alternative pathway is initiated via a number of cell surface molecules other than the antigen receptor. MAb NDA_5 amplifies the direct pathway of activation since in each of four repeat experiments, it augmented, at least twofold, the blastogenic response of purified T cells to soluble anti-CD3 MAb in the presence of adhering cells. MAb NDA_5 also enhanced the response of purified T cells to 12-*O*-tetradecanoylphorbol-13-acetate (TPA)/ionomycin (Fig. 3). Similar to the results obtained in the PWM system, the response to PHA was both accelerated and augmented in the presence of MAb NDA_5, compared with cultures with no antibody or with MAb MOPC 104E (Fig. 3).

To establish whether the amplification effect results from an increase in frequency of cells expressing the receptor for IL-2 or from an increase in the density of this receptor, T cells were stimulated with PHA in the presence or absence of MAb NDA_5 and tested for binding FITC-conjugated MAb anti-CD25 on days 1 and 2 of culture. Both the number of CD25-positive cells and the intensity of fluorescence were similar in cultures with and without MAb NDA_5 (Fig. 4).

Effect of MAb NDA_5 on T cell responses to antigens. To determine whether MAb NDA_5 potentiates primary or secondary T cell responses to soluble antigens, we measured its effect on the blastogenic response of PBMC from healthy staff members to antigens to which they have or have not been sensitized in vivo. As controls we used MAbs MOPC-104E, HeL-1-anti-CD45 and an anti-CD43 MAb kindly provided by Dr. Brent Axellson from the University of Uppsala. All

Table II. Comparison of the Effect of MAb NDA_5 and Its $F(ab')_2$ Fragment on the PWM Response of PBMC from a Healthy Individual

	Day 3 proliferative response to PWM in cultures containing MAbs				
Concn of MAb	NDA_5	NDA_5 $F(ab')_2$	T191	T191 $F(ab')_2$	HEL-1
μg/ml		cpm × 10^3			
0.000	37	37	37	37	37
0.250	82	54	28	30	38
0.500	91	65	22	25	36
1.00	93	74	23	23	29

Mean counts per minute from triplicate wells. Standard errors of the mean were < 10%.

Table III. Dynamics of the Amplification Effect Induced by MAb NDA_5

Reactivity to PWM in cultures	Time when MAb were added	Day 3 blastogenic responses	
		Responder no. 1	Responder no. 2
	h	cpm	
Without MAb		13,496±852	16,070±528
With MAb NDA_5	0	31,762±1,311	26,503±1,712
	4	12,582±924	14,218±676
	17	10,940±648	10,223±359
With MAb MOPC-104E	0	12,768±1,573	13,897±1,237
	4	12,672±1,182	14,005±1,068
	17	10,836±327	12,019±1,006
	42	12,393±1,314	11,058±759
PBMC preincubated with NDA_5		36,557±726	46,996±1,756
PBM preincubated with MOPC-104E		10,309±182	14,590±392

Mean±SD.

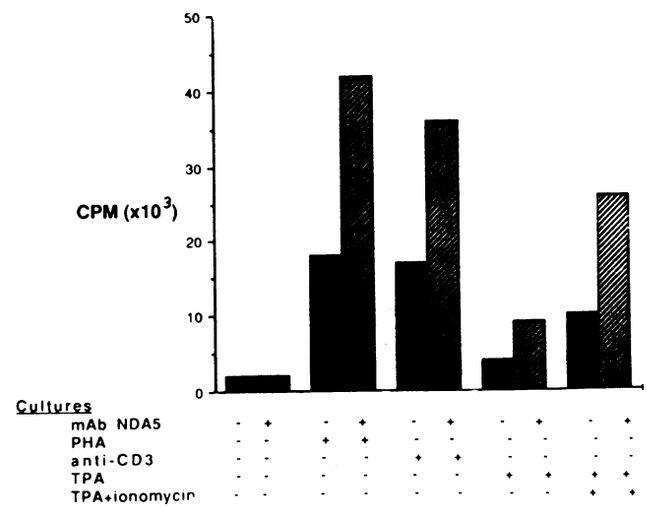

Figure 3. Amplification of T cell responses to anti-CD3 MAb and to phorbol esters by MAb NDA_5. The results are expressed as the mean counts per minute of [^{3}H]TdR incorporation of triplicate cultures. The standard deviation of each value given was < 12% of the mean counts per minute.

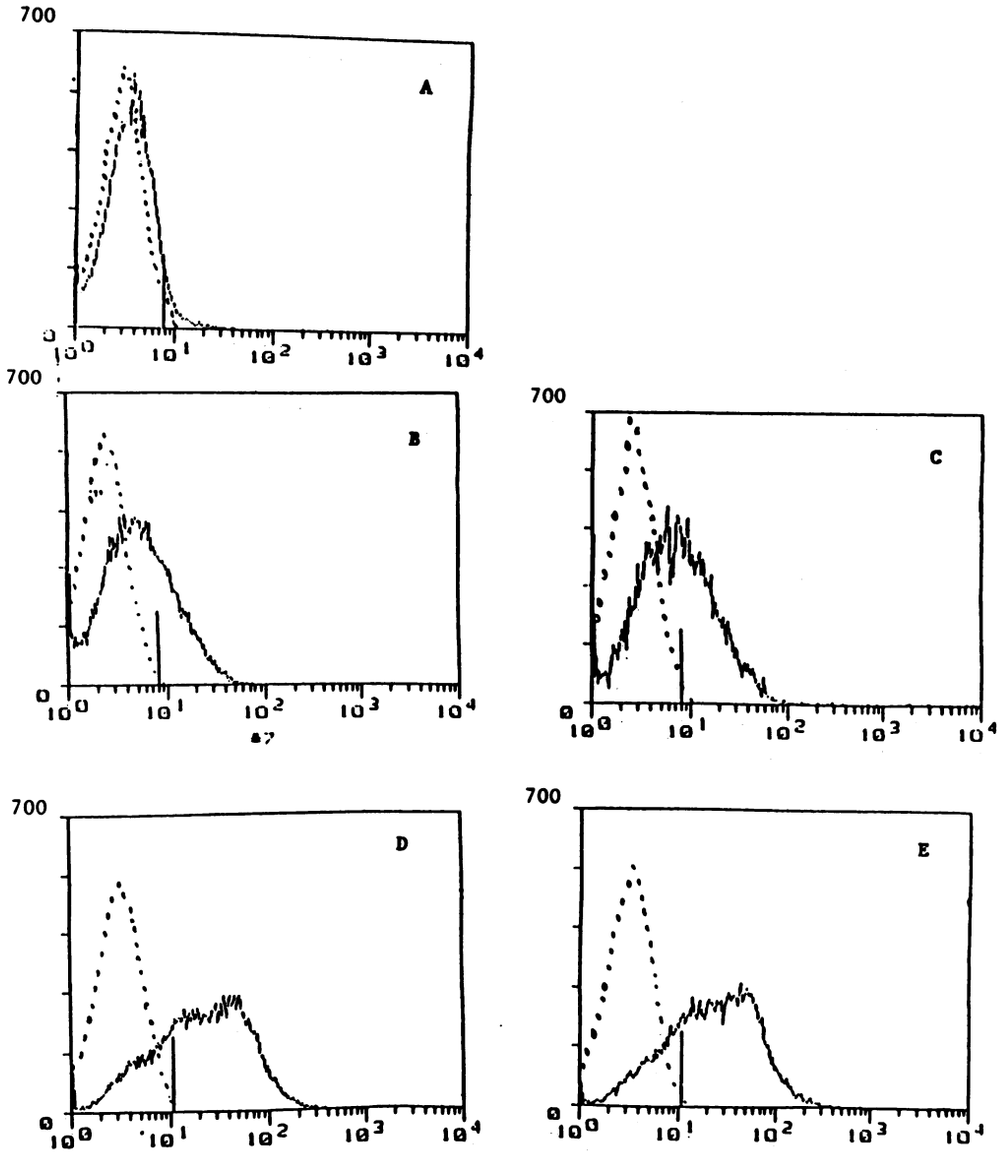

Figure 4. Expression of CD25 (IL-2 receptor) on T cells stimulated with PHA in the presence of MAb NDA_5. IL-2 receptor expression was determined by staining the cells with FITC MAb anti-CD25. (*A*) Before activation; (*B*) after 24 and (*D*) after 48 h of activation in the presence of control MAb MOPC; (*C*) after 24 and (*E*) after 48 h of activation in the presence of MAb NDA_5.

antibodies were tested at a final concentration of 5 μg/ml. Cultures were labeled and harvested after 5 d of incubation. The results of a representative experiment, in a series of six, are shown in Table IV. In the presence of MAb NDA_5, PBMC from both responders showed enhanced blastogenesis in cultures containing tetanus toxoid, PPD, hepatitis B vaccine, and HIV-1 gp-120.

Individual No. 1 had been sensitized to tetanus toxoid and hepatitis B vaccine but has not been vaccinated with PPD or exposed to HIV-1 gp-120. MAb NDA_5 amplified the primary response to PPD and HIV-1 gp-120 as well as the secondary response to tetanus toxoid and hepatitis B. Similarly, the responses of individual no. 2 to tetanus toxoid and PPD with which she had been vaccinated and the response to hepatitis B vaccine and HIV gp-120, to which she has not been exposed, were increased in the presence of MAb NDA_5. The primary response to antigens not used for vaccination, (such as PPD in the case of responder no. 1 and hepatitis B in the case of responder no. 2) was negligible in the absence of any MAbs and in cultures with MAbs MOPC-104E, HeL-1 anti-CD45, or anti-CD43, an antibody known for some of its enhancing effects on blastogenic responses (4). However, under these culture conditions, the blastogenic response to HIV-1 gp-120 could still be detected in both these individuals.

Since this data suggested that MAb NDA_5 augmented both primary and secondary in vitro responses, we explored the possibility of using MAb NDA_5 for amplifying the reactivity of T lymphocytes from healthy individuals to synthetic peptides corresponding to different amino acid sequences of HIV-1 gp-120. Hepatitis B vaccine was included as a control for secondary response and PPD for a primary response. The in vitro blastogenic response was quantitated on days 3, 5, and 7. Fig. 5 illustrates the kinetics of the response exhibited by a healthy women volunteer to three different peptides, hepatitis B vaccine, and PPD. From day 3 to 7 there was a progressive increase of the magnitude of the "primary" response to PPD, HIV-1 gp-120 and to the synthetic peptides 438–466 and 425–452 in cultures containing MAb NDA_5. However, there

Table IV. MAb NDA_5 Augments the Response of PBMC to Soluble Antigens

Responder	MAb added to the culture	Proliferative response				
		Medium	Tetanus toxoid	PPD	Hepatitis B	HIV-1 gp-120
				cpm		
	None	452	48,370	3,780	27,525	16,784
	NDA_5	575	96,510	16,435	54,377	45,263
1	MOPC-104E	580	51,210	3,548	26,832	12,549
	HeL-1 Anti-CD45	428	37,540	1,510	15,428	13,540
	Anti-CD43	620	46,215	2,210	25,412	17,055
	None	1,520	37,523	25,926	2,360	24,756
	NDA_5	815	85,920	62,756	10,482	35,720
2	MOPC-104E	2,210	44,826	33,415	1,782	20,212
	HEL-1 Anti-CD45	388	25,340	14,578	1,214	18,476
	Anti-CD43	640	28,740	38,420	1,726	26,840

Values are means.

was no primary reactivity to peptides 477–508. In control cultures with MOPC-104E there was no primary reactivity to PPD and only borderline reactivity to one of the peptides, although there was a progressive increase in the reactivity to HIV-1 gp-120. The "secondary" response to hepatitis B vaccine, with which this women had been immunized, increased rapidly from day 3 to 5 and plateaued on day 7, in the absence of MAb NDA_5. In cultures with MAb NDA_5 a much higher peak was reached on day 5 after which the response declined in a pattern characteristic of a secondary response. The kinetics of these responses are consistent with the possibility that MAb NDA_5 amplifies both primary and secondary T cells responses.

We next established whether MAb NDA_5 enhances the response of patients with AIDS to HIV-1 gp-120. In these experiments cryopreserved PBM (1×10^5 cells) from two AIDS patients and two healthy controls were stimulated in triplicate wells of 96-well trays with HIV-1 gp-120 or tetanus toxoid, in the presence or in the absence of MAb NDA_5 (Table V). To increase the number of antigen presenting cells, irradiated (3,000 rads) HLA-D homozygous lymphoblastoid B cell lines (HTC-LBCL) (5×10^4 cells per well) expressing one of the responder's HLA-D antigens were added to the cultures. The HLA phenotype of patients, controls, and LBCL was characterized by serologic typing to insure compatibility between responding cells and HTC-LBCL for HLA-DR and DQ antigens.

In the presence of MAb NDA_5 there was augmentation of the response exhibited by PBMC from healthy individuals to HIV-1 gp-120 and to tetanus toxoid. This amplification effect was observed both in cultures with and without HTC-LBCL.

In cultures without MAb NDA_5, PBMC from AIDS patients showed no detectable reactivity to HIV-1 gp-120 or tetanus toxoid, even when HTC-LBCL were present. However, addition of MAb NDA_5 to cultures containing soluble antigens and HTC-LBCL enhanced the blastogenic responses above the levels seen in cultures in which the patients' PBM were exposed to HTC-LBCL only.

Since the patients reactivity to HIV-1 gp-120 and tetanus toxoid could not be evidenced in the absence of MAb NDA_5, this antibody may enhance reactivity to soluble antigens by increasing the contact between responding T cells and antigen presenting cells (Table V).

The possibility of enhancing memory responses which have been induced in vitro was explored using the primed lymphocyte test. For this, PBM from an HLA-DW4/DW6 heterozygous individual were stimulated in primary MLC with an equal number of irradiated PBMC from a DW2/DW4 heterozygote. Small T lymphocytes were collected after 10 d and rechallenged in secondary cultures with irradiated cells from the specific, primary stimulator and from controls with different HLA-D/DR antigens. The memory response to the specific stimulator used for primary in vitro sensitization was significantly higher in the presence of MAb NDA_5, than in cultures without antibody or with MAb MOPC-104E. The potentiating effect of MAb NDA_5 was already evident after 24 h and could still be seen after 48 and 72 h (Table VI). As expected for primary in vitro allorecognition, the response to the DW2-negative control showed a slower progression. The enhancing effect of MAb NDA_5 on this primary reaction, became detectable only after 72 h. The primed lymphocytes maintained the ability to discriminate between specific and control stimulators in the presence of MAb NDA_5, suggesting that this antibody acted specifically on the lymphocyte clones engaged in the memory response. Thus, virgin as well as memory T lymphocytes are susceptible to the potentiating effect of MAb NDA_5 when stimulated with allogeneic HLA-D/DR antigens.

To determine whether MAb NDA_5 stimulates the proliferation of T lymphoblasts in the absence of any antigen, we added the antibody to IL-2–dependent normal and malignant T cell clones and to IL-2–independent leukemic T cells lines (CEM). No increase in the rate of T cell proliferation was observed in the presence of MAb NDA_5, after 24, 48, or 72 h of cultures regardless of the density at which the cells were seeded and of the concentration of IL-2 added to the cultures (data not shown).

Discussion

Recently we described two new differentiation antigens, NDA_3 and NDA_4, which are recognized by MAbs that were obtained

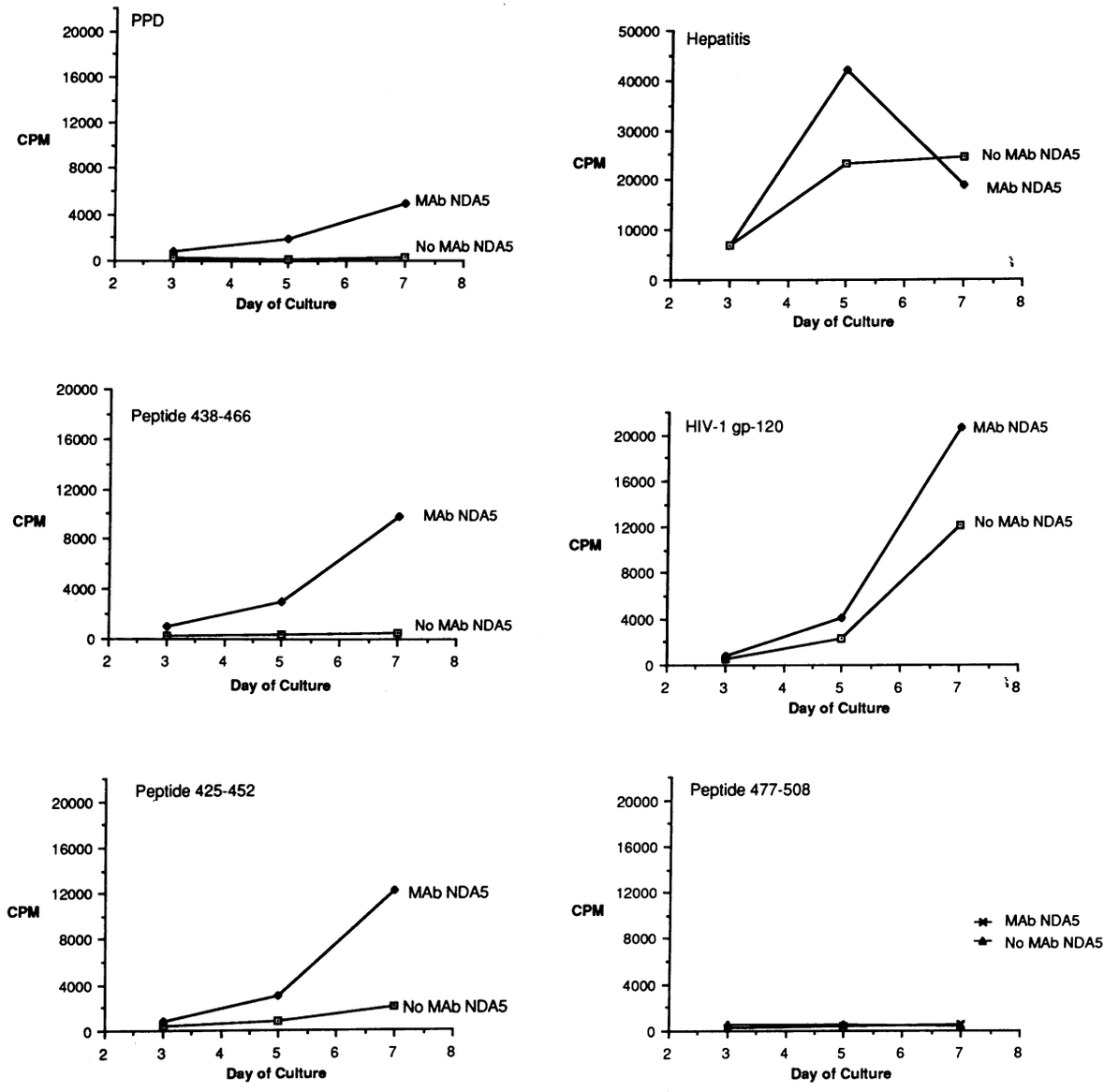

Figure 5. Time course of the blastogenic response of PBMC from a normal individual to soluble antigens. PBMC were cultured for the indicated periods in the presence of soluble antigens with or without MAb NDA_5. Cultures were labeled with [^{3}H]TdR 18 h before harvesting. Values given are the mean counts per minute from triplicate wells. The standard deviation at each point was < 15% of the mean counts per minute.

after immunization of mice with an alloreactive helper T cell clone. These MAbs were shown to enhance the proliferation and maturation of activated B lymphocytes (28). Here, we have defined the immunoreactivity and functional attributes of MAb NDA_5 which recognizes an epitope of CD45, the LCA.

MAb NDA_5 was also generated by immunizing mice with a human T cell clone and is endowed with the capacity of amplifying T cell proliferative responses to soluble antigens, alloantigens, and plant mitogens. The MAb NDA_5, an IgM, binds strongly to the cell membrane as established by immunofluorescence, aggregation, and functional studies, yet it seems unable to recognize the detergent-solubilized form of the antigen during immunoprecipitation. This, in conjunction with the fact that many IgM MAbs recognize tertiary structures, which may be lost during solubilization, hampers the immunoprecipitation of the NDA_5 molecule. The reactivity of this antibody with mouse cell lines transfected with the individual isoforms of the LCA family, however, has provided strong evidence that MAb NDA_5 belongs to the CD45 cluster of differentiation.

Some antibodies to the CD43 and CD45 molecules were shown to amplify the expression of the IL-2 receptor, thereby augmenting T cell responses to mitogens and specific antigens (3). Although MAb NDA_5 also enhances T cell responses, immunofluorescence studies of lymphocytes stimulated with PHA in the presence of this MAb showed no increase in the level of IL-2 receptor expression. This contrasts the characteristics of previously reported anti–CD45 MAbs which regulate IL-2 receptor expression on PBMC and/or enhance CD3-driven T cell proliferation only in the presence of exogenous IL-2 (12, 17). The IL-2 receptor-independent action of MAb NDA_5 suggests that alternate intracellular signal transmission events also participate in T cell blastogenesis.

The mechanism involved in the amplification effect occur-

Table V. Amplification of T Cell Responses to HIV-1 gp-120 in Healthy Individuals and in AIDS Patients with Kaposi's Sarcoma

Responders	MAb NDA_5 added	Medium	Reactivity				
			HTC-LBCL	HIV-1 gp-120	HIV-1 gp-120+ HTC-LBCL	Tetanus Toxoid	Tetanus + HTC-LBCL
			cpm × 10^3				
Control 1	No	0.8	46	0.5	46	2	53
	Yes	0.4	49	11	64	12	65
Control 2	No	0.3	42	3	71	10	69
	Yes	0.2	62	9	93	27	110
Patient 1	No	0.2	8	0.2	7	0.2	10
	Yes	0.2	11	0.2	22	0.2	16
Patient 2	No	0.1	15	0.1	11	0.1	14
	Yes	0.3	26	0.4	54	3	47

Patient 1 had 16% T4 and 39% T8 at the time of testing. Patient 2 had 18% T4 and 36% T8 at the time of testing. Values are means.

ring when lymphocytes were activated with soluble antigens, alloantigens, or plant mitogens in the presence of MAb NDA_5 is not yet clear. Accelerated and highly augmented T cell responses were observed if cells were preincubated with MAb NDA_5 or if the MAb was added at the same time as the stimulating agent. Since the amplification effect was dependent on the exposure of responding cells to MAb NDA_5 at the time of activation, and did not occur if the antibody was added 4–24 h later, this antibody seems to act during early stages of T cell triggering. Similar co-stimulatory effects have been described for monokines and lymphokines which potentiate activation signals induced by cross-linking the antigen specific receptors with anti-CD3 or anti-μ antibodies, respectively (29–31). However, as opposed to lymphokines, or to anti-lymphokine receptor antibodies (which may mimic the effect of their ligand), MAb NDA_5 did not stimulate the growth of T cell lines or of already activated, MLC-primed human T cells, unless they were restimulated with the specific HLA-D/DR antigen(s). This suggests that MAb NDA_5 amplifies antigen-specific T cell responses. Since MAb NDA_5 amplifies T cell activation by anti-CD3 MAb as well as by TPA/ionomycin, this MAb may act at a point common to the antigen and alternative pathways.

Our findings reinforce the concept that LCA plays an important role in signal transduction events that initiate the immune response. This concept is supported by previous studies showing that CD45 MAbs, when coupled to CD4 MAb, can induce an increase in intracellular calcium concentration (6) and thus provide a positive proliferative signal. The potentiating effects of MAb NDA_5, which contrasts the inhibitory effects of most anti-CD45 MAb, does not appear to be related to the IgM isotype of MAb NDA_5; the $F(Ab')_2$ fragment of this antibody is also stimulatory in PWM-triggered proliferation assays.

It is very likely, however, that steric influences (32, 33) play an important role in determining the enhancing effect of MAb NDA_5. MAb NDA_5 may induce a particular geometry of the LCA expressed on the cell membrane, facilitating the initial signaling events, possibly by sequestering the protein tyrosine phosphate phosphatase activity associated with CD45 (34). At least two other anti-CD45 IgM MAbs have been studied. These IgMs, however, inhibit the blastogenic response of PBM to soluble antigens (Morimoto, C, personal communication). Thus, the effects of anti-CD45 MAb on lymphocyte blastogenesis is not necessarily related to their isotype.

It is also possible that certain epitopes of the CD45 molecule play a role as cell adhesion structures with immunoregulatory functions. This hypothesis is consistent with the observation that MAb NDA_5 potentiates blastogenic responses induced by antigens or mitogens in the presence of APC. The amplification effect may reflect increased heterotypic interactions between responding T cells and cytokine producing cells. Supporting the possibility that MAb NDA_5 strengthens cell–cell interactions which may control T cell activation is the fact that this antibody induces the formation of aggregates between cells of the same and of different (T and B) compartments after 18 h of incubation.

Among other mechanisms of action, the possibility that MAb NDA_5 inhibits in some manner the generation or function of suppressor cells should also be considered. Proof to this effect, however, requires the identification and characterization of such a suppressor population. Since the antigen recognized by MAb NDA_5 is present on both CD4 and CD8 positive cells, and since there is functional heterogeneity within each subset, phenotyping for these markers does not provide an answer to this question.

Table VI. Secondary Responses of Alloreactivated T cells Amplified by MAb NDA_5

Responding cells: anti-DW2 primed lymphocytes	Cells used for secondary priming						
		Specific stimulator: HLA-DW2, DW4			Control: HLA-DW3, DW4		
Priming period (*h*)	None 72	24	48	72	24	48	72
	cpm × 10^3						
Without MAb	2	28	47	80	8	15	15
With MAb NDA_5	1	48	73	130	8	15	35
With MOPC-104E	2	23	46	85	7	13	24

We have demonstrated the MAb NDA_5 enhances the primary in vitro response to PPD, HIV-1 gp-120, and derived peptides in normal individuals. PBMC from HIV-infected individuals are also susceptible to the enhancing effects of MAb NDA_5. In the presence of MAb NDA_5, the blastogenic response of T lymphocytes from these patients to HIV-1 gp-120 was significantly increased, suggesting that the signal transducing mechanisms in these cells are still functional. The possibility exist that HIV infection of T cells alters the ability of LCA to efficiently transduce proliferation signals, yet this alteration may be reversed in the presence of MAb NDA_5.

Regardless of the mechanism involved in the amplification effect displayed by MAb NDA_5, its efficiency in stimulating the proliferative response of lymphocytes from AIDS patients is of particular importance. The fact that many patients' responsiveness was greatly enhanced, in spite of their inverted CD4/CD8 ratio, suggests that their depressed immunoreactivity may be manipulated in a clinically beneficial manner. Attempts to vaccinate immunologically deficient patients against viral or bacterial antigens might have a better chance of success if the generation of effector cells from resting precursors could be enhanced. This could be accomplished by increasing T cell adhesion to antigen-presenting cells and/or intra and intercellular transmission of activation signals. In this context, our observation that MAb NDA_5 enhances specific immune responses raises the possibility of using such reagents as adjuvants for active immunization procedures.

The finding that MAb NDA_5 amplifies primary and secondary T cell responses to a variety of antigens such as HIV-1 gp-120 presents the opportunity of studying cognitive responses to T cell epitopes of HIV with low immunogenicity.

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