

Immunodeficiency and other clinical immunology

CD45 isoforms on human CD4⁺ T-cell subsets

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We have examined the level of surface expression and functional properties of leukocyte function associated antigen-1, very late antigen-4, and CD45 isoforms on a panel of human CD4⁺ T-cell clones representative of TH₀, TH₁, and TH₂ cells. There were no qualitative differences in the expression of these antigens among the three types of CD4⁺ T-cell clones. However, CD45RB was the only CD45 isoform that provided a costimulatory signal in a solid-phase antibody-induced cellular proliferation assay. Additionally, the antigen-induced proliferative response of T-cell clones was inhibited by soluble anti-CD45RO and anti-CD45RB antibodies. Our results suggest that CD45 isoforms differentially provide costimulatory signals to T cells. However, the ability of each CD45 isoform to provide a costimulatory signal does not differ among the TH₀, TH₁, or TH₂ T-cell populations. (J Allergy Clin Immunol 1996;98:433-40.)

Key words: CD45 isoforms, T-cell subsets, costimulatory molecules

CD4⁺ T cells can be subdivided into functionally distinct subsets, on the basis of the profile of cytokines produced after stimulation. One subset, referred to as type 1 helper T (TH₁) cells, produces interferon- γ and IL-2 on activation and mediates delayed-type hypersensitivity. A second subset, type 2 helper T (TH₂) cells secretes IL-4, IL-5, and IL-10 and supports antibody production by B cells.¹ There is at least one additional CD4⁺ T-cell subset, referred to as TH₀ cells, which produces

Abbreviations used

LFA: Leukocyte function associated antigen
mAb: Monoclonal antibody
VLA: Very late antigen

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cytokines characteristic of TH₁ and TH₂ cells.² Analysis of the cellular immune responses to infectious pathogens in experimentally infected animals has indicated that pathologic inflammation may be mediated through the selective activation of CD4⁺ T-cell subsets with distinct patterns of cytokine production.^{1,3,4} Interest in this phenomenon increased when the selective activation of helper T cells with a cytokine profile similar to that of TH₁ or TH₂ cells was subsequently noted in human chronic inflammatory and allergic diseases, respectively.⁵

It is of great importance to understand the mechanism by which the profile of cytokines produced by CD4⁺ T cells is imprinted and to understand why a particular CD4⁺ T-cell subset will dominate an immune response in vivo. Essential to this is the identification of a biochemical marker, which distinguishes among the CD4⁺ T-cell sub-

TABLE I. Characteristics of T-cell clones used in this study

Donors	T-cell clones	Specificity	Cytokine production profile	Reference
Lyme disease	CR-253/378/380	<i>B. burgdorferi</i>	TH ₁	26
Lyme disease	AP-75/141	<i>B. burgdorferi</i>	TH ₁	26
Lyme disease	RF-141/244/262	<i>B. burgdorferi</i>	TH ₁	27
	2RF-48/58/88/93	<i>B. burgdorferi</i>	TH ₁	27
Atopic	DA-26/78/245	Cat allergen	TH ₁	This study
Atopic	DA32	Cat allergen	TH ₀	This study
Atopic	DA-4	Cat allergen	TH ₂	This study
Atopic	AD128	<i>Dermatophagoides pteronyssinus</i>	TH ₂	28
Atopic	NP-12/38	<i>D. pteronyssinus</i>	TH ₂	28

TABLE II. Profile of cytokine production by T-cell clones isolated from patient DA

T-cell clones	Interferon- γ (ng/ml)	IL-4 (ng/ml)
DA4	0.2	>15
DA26	11.2	<0.1
DA32	33.3	6.1
DA78	12.2	<0.1
DA245	11.6	2.0

T-cell supernatants for lymphokine assays were prepared as described in Methods.

sets. A number of different factors have been implicated in the selective activation of TH₁ or TH₂ cells, including the type of antigen-presenting cell,⁶ steroid hormones,⁷ and intracellular cyclic adenosine monophosphate concentration.⁸ Furthermore, evidence has indicated that human, rat, and murine CD4⁺ T-cell subsets with distinct profiles of cytokine production differentially express isoforms of the CD45 membrane glycoprotein.⁹⁻¹³ The cell type and activation-dependent expression of different protein isoforms of CD45 are determined by alternative splicing of three consecutive NH₂-terminal exons (referred to as A, B, and C) of the CD45 transcript.^{14,15} CD45 plays a major role in regulating signal transduction through the CD3/T-cell antigen receptor complex of T cells,¹⁶ possibly by isoform-specific association with cell surface molecules involved in signal transduction^{10,17} and through regulation of tyrosine phosphorylation of intracellular proteins on T-cell activation.¹⁸ The cytoplasmic domain of CD45 has a tyrosine phosphatase activity,¹⁹ which is the major tyrosine phosphatase activity associated with T-cell membranes¹⁹ and has been shown to acti-

vate the tyrosine kinase activity of p56^{lck20} and p59^{lyn}.²¹ In addition, heterogeneity at the level of cell surface expression of other cellular adhesion molecules, including leukocyte function associated antigen (LFA)-1 and very late antigen (VLA)-4²⁴ on subsets of human CD4⁺ T cells has also been described.

In this article, we have compared the level of expression and functional properties of CD45 isoforms and other cell surface molecules involved in T-cell activation or homing on a panel of human T-cell clones representative of TH₀, TH₁, and TH₂ T cells.

METHODS

T-cell clone generation and characterization

T-cell clones (isolated from patients AP, CR, RF, AD, and NP), producing cytokines characteristic of TH₁, TH₂, and TH₀ cells, used in this study are described in Table I²⁵⁻²⁷ and in the indicated references. Peripheral blood mononuclear cells obtained from donor DA, an individual allergic to cat antigens, were incubated with 1000 U/ml cat antigen, (4803u4; Miles Pharmaceuticals, Spokane, Wash.) for 7 days in vitro, and T-cell clones were prepared according to previously described procedures.^{25,28} Culture supernatants were prepared from 20-hour cultures of concanavalin A-activated T-cell clones, and the presence of various cytokines was determined by using cytokine-specific ELISAs, as described previously.^{25,28} The production of IL-4 or interferon- γ (in nanograms per milliliter) by the T-cell clones DA4, DA26, DA32, DA78, DA245 is indicated in Table II. Analysis of cell surface antigen expression was performed by flow cytometry with a FACScan (Becton Dickinson & Co., Mountain View, Calif.), as previously described.^{25,28} Monoclonal antibodies (mAbs) against the following antigens (clones indicated in parentheses) were used both in flow cytometry and proliferation assays: CD45RO (UCHL1) and CD45RB (PD7/26) from Dako (Denmark); CD45RA (Leu 18; kindly pro-

vided by Dr. Lewis Lanier, DNAX, Palo Alto, Calif.); and CD45RA (ALB11), LFA-1 (25.3), VLA-4 (HP2/1), and CD3 (X35) from AMAC (Westbrook, Maine). In addition, mAbs to the following cell surface molecules were used in flow cytometry: CD28 (CD28.2), CD31 (5.6E), HML-1 ($\alpha\epsilon\beta 7$; 2G5), CD11b (Bear1), CD11c (BU15), VLA-2 (CDw49b or $\alpha 2\beta 1$, Gi9), VLA-5 (CDw49e or $\alpha 5\beta 1$; SAM1), VLA-6 (CDw49f or $\alpha 6\beta 1$; GoH3), LFA-3 (AICD58) from AMAC; CD44 (Hermes-3; kindly provided by Dr. Sirpa Jalkanen, National Public Health Institute, Turku, Finland); cutaneous lymphocyte antigen (HECA 452, kindly provided by Dr. Eugene Butcher, Stanford University); Act-1 ($\alpha 4\beta 1$; kindly provided by Dr. Steven Shaw, National Institutes of Health, Bethesda Md.), L-selectin (Leu-8), intercellular adhesion molecule-1 (CD54, L142), B7 (L310), CD7 (Leu9), CD15 (LeuM1), CD16 (Leu 11a), CD43 (L60), and CD56 (L185) (all kindly provided by Dr. Lewis Lanier, DNAX). In addition, a panel of 85 antibodies included in the V International Workshop on Human Leukocyte Differentiation Antigens (1993) was used.

Proliferation assays

Antigen-specific cellular proliferation was determined by incubation of the indicated T-cell clone with antigen (*Borrelia burgdorferi* sonicate) and autologous B lymphoblastoid cells (as antigen-presenting cells) for 72 hours, and measuring the amount of tritiated thymidine incorporated over the last 6 hours.²⁵ For antibody inhibition studies, T-cell clones were incubated with 5 $\mu\text{g}/\text{ml}$ of the indicated mAb at 37° C for 1 hour before assay. For solid-phase proliferation assays, 50 μl of 10 $\mu\text{g}/\text{ml}$ solution of goat anti-mouse antibodies (AU272; The Binding Site, Birmingham, England) in 15 mmol/L sodium carbonate (pH 9.6) buffer were incubated in round-bottom microwells (Flow Laboratories, McLean, Va.) overnight at 4° C. The plates were then saturated with a solution of Iscove medium supplemented with 3% bovine serum albumin. After removal of this solution, a 3% bovine serum albumin–Iscove's medium preparation containing 2 mg/ml murine antibodies recognizing the indicated human T-cell antigens, was incubated in the microwells for 2 hours at 37° C. The microwells were then rinsed twice before addition of 2.5 to 4 $\times 10^4$ cells of a T-cell clone. Cellular proliferation was determined by measuring tritiated thymidine incorporation during the last 6 hours of a 72-hour incubation in the antibody-coated wells.

RESULTS

The level of expression of multiple antigens on the cell surface of 15 TH₁ clones isolated from one atopic patient and three patients with Lyme disease, four TH₂ clones isolated from three atopic patients, and one TH₀ clone (described in Table I) was determined by flow cytometry (representative data in Fig. 1). All T-cell clones expressed quali-

tatively similar amounts of VLA-4, LFA-1, and three isoforms of CD45 (CD45RO, -RA, and -RB). The relative expression of the CD45RO and CD45RB antigens was variable among T-cell clones but consistently higher than the surface expression of the CD45RA molecules. The expression of low but detectable amounts of CD45RA was confirmed by using two different CD45RA-specific mAbs. The profile of CD45 isoform expression on a given clone was stable when analyzed after different numbers of in vitro restimulations and did not differ with time after activation (data not shown). In addition, comparable levels of 20 other cell surface antigens were expressed by TH₁- or TH₂-like T-cell clones. These cell surface molecules included VLA-2 ($\alpha 2\beta 1$), VLA-5 ($\alpha 5\beta 1$), VLA-6 ($\alpha 6\beta 1$), HML-1 ($\alpha\epsilon\beta 7$), Act-1 ($\alpha 4\beta 1$), cutaneous lymphocyte antigen, CD44, L-selectin, B7, LFA-3, intercellular adhesion molecule-1, CD11b, CD11c, CD7, CD15, CD16, CD28, CD31, CD43, and CD56. Furthermore, none of the 85 mAbs included in the V International Workshop on Human Leukocyte Differentiation Antigens (1993) was able to distinguish between our TH₁- and TH₂-like T-cell clones.

Although CD4⁺ T-cell clones with different profiles of cytokine production expressed similar levels of the cell surface molecules examined, it was possible that they provided functionally distinct activating signals to the different types of T cells. To investigate this, the ability of a particular cell surface antigen to provide a costimulatory signal triggering cellular proliferation was assessed. The proliferative responses of T-cell clones after a 72 hour incubation with antibodies recognizing CD3, LFA-1, VLA-4, CD45 isoforms, or a combination of these antibodies were measured (Fig. 2). None of the antibodies by themselves, including an anti-CD3 antibody, induced significant proliferative responses in any of the T-cell clones tested (Fig. 2 and data not shown). However, the combination of the anti-CD3 antibody with either anti-LFA-1 or anti-CD45RB antibodies induced significant proliferative responses in all of the T-cell clones tested, irrespective of their profile of cytokine production (Fig. 2). In contrast, anti-VLA-4, anti-CD45RA, or anti-CD45RO antibodies provided little or no costimulatory signal to the T-cell clones when combined with anti-CD3. Cross-linking of CD45 isoforms did not consistently modify the proliferative response induced by stimulation through CD3 and LFA-1 or CD3 and VLA-4, respectively. For example, the proliferative responses of only two of 17 T-cell clones tested

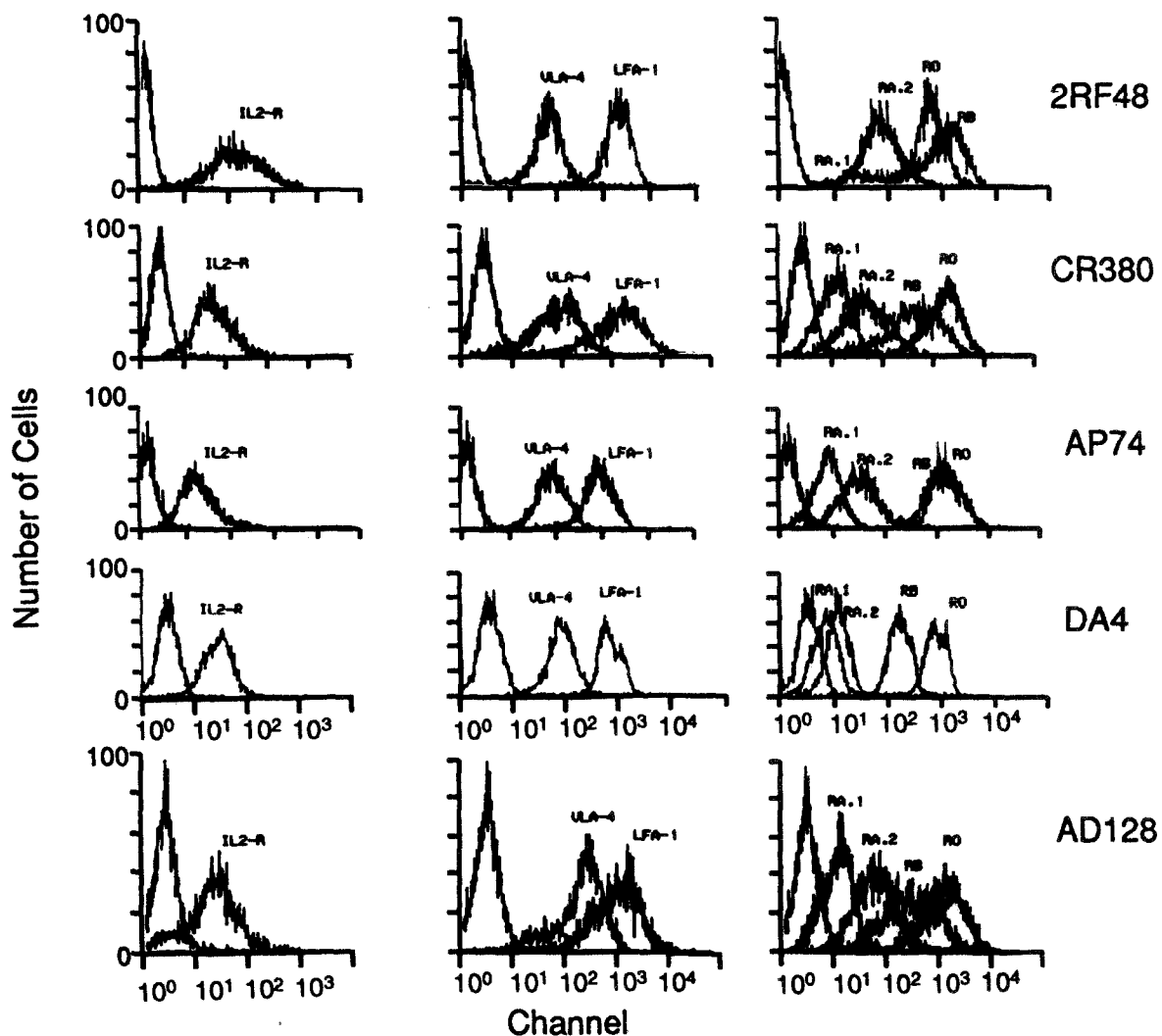


FIG. 1. Cell surface antigens of human CD4⁺ T-cell clones. The cell surface expression of isoforms of CD45, VLA-4, LFA-1, and IL-2 receptor on human CD4⁺ T-cell clones with TH₁-like (2RF48, CR380, and AP74) or TH₂-like (DA4 and AD128) profiles of cytokine production was determined by flow cytometry.

(RF262 and 2RF93) increased (twofold) when VLA-4 was cross-linked in addition to CD3 and CD45RB (Fig. 2). Similarly, proliferative responses of only three of 17 T-cell clones tested (CR380, 2RF93, and AP75) increased when CD45RA was cross-linked in addition to CD3 and LFA-1 (Fig. 2).

To functionally assess the significance of the costimulatory signals provided to T-cell clones by the integrins and CD45 isoforms examined previously, the ability of soluble antibodies to inhibit the antigen-specific proliferative response of T-cell clones was examined. Soluble anti-CD3, anti-CD45RO, or anti-CD45RB antibodies significantly inhibited proliferative responses of three T-cell clones examined (RF244, RF88 and 2RF99) to

B. burgdorferi antigen presented by autologous Epstein-Barr virus-transformed B lymphoblastoid cells (representative data in Fig. 3). In contrast, soluble anti-LFA-1, anti-VLA-4, or anti-CD45RA antibodies did not inhibit antigen-specific proliferative response of the T-cell clones (Fig. 3 and data not shown). The addition of combinations of antibodies binding to different CD45 isoforms did not increase the extent of inhibition of antigen-specific T-cell proliferation (e.g., Fig. 3, clone RF244). We also compared the ability of soluble anti-CD45 antibodies to inhibit the superantigen-induced (SEB or SEE, depending on the T-cell antigen receptor V β chain expressed by a given T-cell clone) proliferation of a panel of TH₁ or TH₂ cells. Consistent with results shown in Fig. 3, antibodies

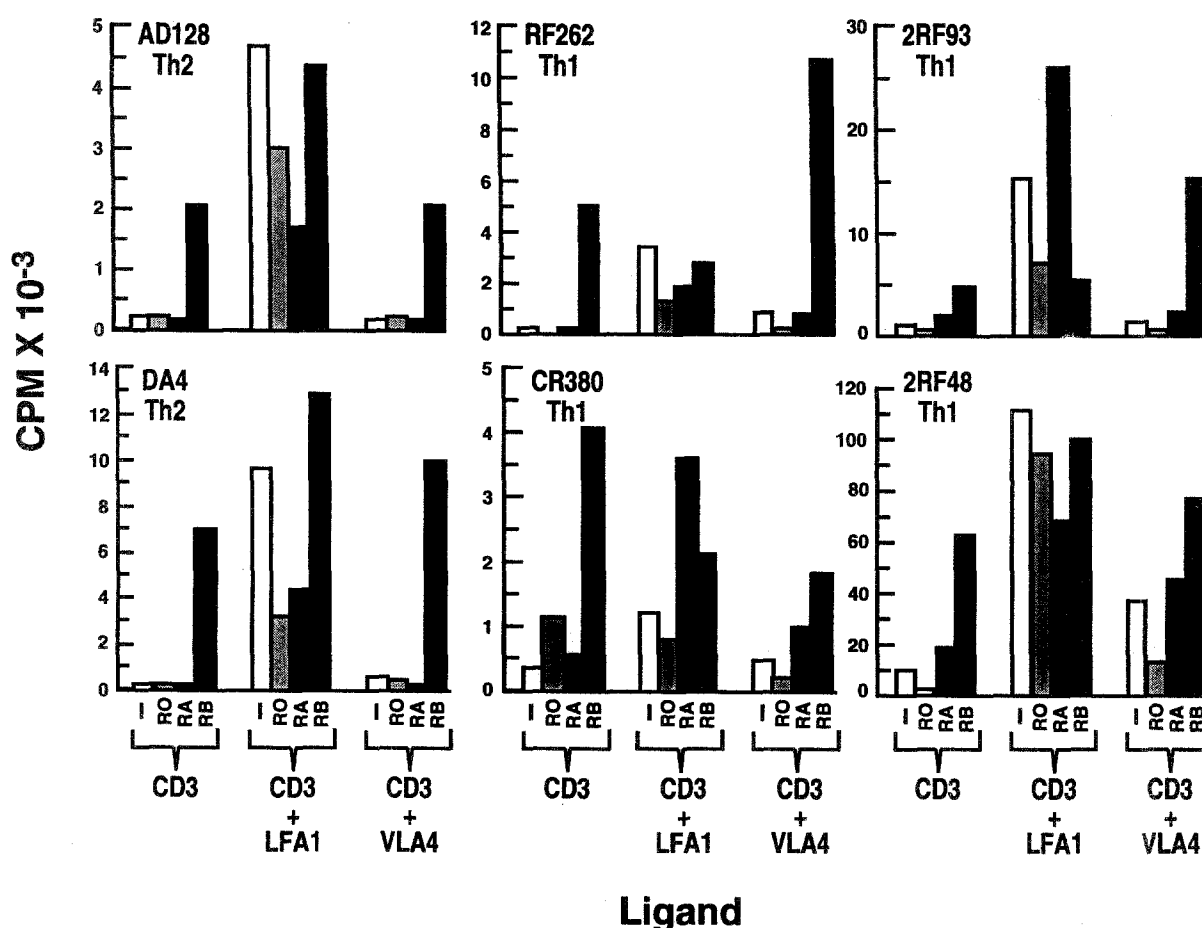


FIG. 2. Solid-phase antibody-induced proliferation of CD4⁺ T-cell clones. The proliferative response of T-cell clones after a 72-hour incubation in microwells coated with combinations of mAbs recognizing the indicated antigens was determined by measuring tritiated thymidine incorporation. Each bar represents the mean of three individual determinations, and the standard deviation was less than 20%.

to CD45RO or CD45RB inhibited superantigen-induced proliferation of T-cell clones representative of either T-cell subset (data not shown).

DISCUSSION

In this study we measured the level of surface expression and characterized functional properties of the surface molecules LFA-1, VLA-4, CD45RO, CD45RA, CD45RB on human T-cell clones. Fifteen TH₁-, one TH₀- and four TH₂-like human T-cell clones were considered in the experiments. The cloned T cells had maintained their profile of cytokine production after in vitro culture, an essential biochemical property distinguishing TH₁ and TH₂ cells; therefore, they served as valid representatives of human CD4⁺ T-cell subsets for experimental purposes.

We were quite surprised to find that our panel of human CD4⁺ T-cell clones expressed quantita-

tively similar amounts of the costimulatory molecules examined on their cell surface and that they abundantly expressed both low (CD45RO) and high molecular weight (CD45RB) isoforms of CD45. It had previously been reported that mouse^{12,13} and human^{9,10} "naïve" CD4⁺ T-cell populations express CD45RA and CD45RB, whereas "memory" CD4⁺ T cells specifically express the CD45RO isoform. A number of other experimental observations also suggested that human TH₁- and TH₂-like cells would express different costimulatory molecules. Heterogeneity in the amount of cellular adhesion molecules, including LFA-1, expressed on human CD4⁺ T cells, which mediate costimulation had been noted^{22,23}; and the level of expression of several adhesion molecules, including LFA-1 and CD45RO, correlated with the amount of interferon- γ secreted on stimulation.²² Magilavy et al.⁶ reported the preferential

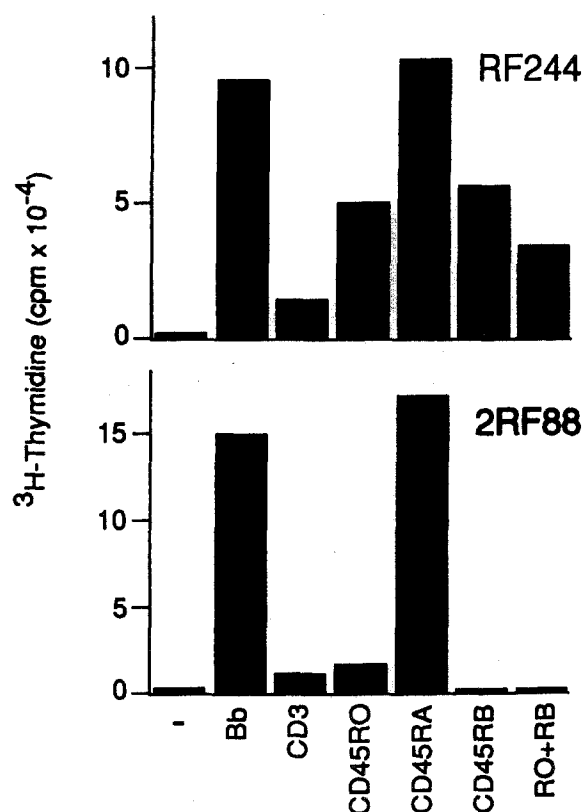


FIG. 3. Anti-CD45 antibodies inhibit the antigen-induced proliferative response of human CD4⁺ T-cell clones. Two CD4⁺ T-cell clones (RF244 and 2RF88) proliferated in response to *B. burgdorferi* (Bb) antigen, presented by an autologous Epstein-Barr virus-transformed B lymphoblastoid cell line, and did not proliferate in the presence of the B-cell line alone (-). Preincubation of these T-cell clones with mAbs recognizing CD3, CD45RB, or CD45RO, but not CD45RA, inhibited their proliferative response. Cellular proliferation was measured as in Fig. 2. Each bar represents the average of three individual determinations, and the standard deviation was less than 20%. These results are representative of the finding obtained with nine different TH₁-like T-cell clones, *B. burgdorferi*-specific.

stimulation of murine TH₁ and TH₂ clones in response to stimulation with antigen presented by different types of antigen-presenting cells,⁶ suggesting that they have different costimulatory ligand requirements. Most relevant was the observation that murine CD4⁺ TH₁ and TH₂ clones differentially expressed CD45 isoforms; murine TH₂ cells preferentially expressed high molecular weight CD45 isoforms not expressed on TH₁ cells.^{29,30} However, our finding that the RO and RB isoforms of CD45 are simultaneously expressed on clonal populations of human CD4⁺ T cells is consistent with the pattern of CD45 isoform-specific messenger RNA expression noted in

human CD4⁺ T cells and murine antigen-specific TH cell lines.¹⁴ The predominant transcripts found in resting and phorbol myristate acetate-activated CD4⁺CD45RO⁺ T cells prepared from human peripheral blood mononuclear cells encode CD45RO and CD45RB isoforms. Although murine TH₂ cells expressed more CD45 mRNA containing exon B than TH₁ cells, CD45RB mRNA was present in both TH₁ and TH₂ clones. Furthermore, the most abundant CD45 transcript in TH₁ and TH₂ murine T-cell clones was CD45RO.¹⁴ In addition, evidence that human T-cell clones can simultaneously express at least two distinct CD45 isoforms has been previously presented.³¹ A small number of human T-cell clones, which express the CD45RO isoform at rest and CD45RO and CD45RA isoforms after activation, have been previously characterized.³¹ Therefore it appears that human TH₁ and TH₂ cells express both RO and RB isoforms of CD45 and do not differentially express CD45 isoforms.

The results of the antibody-induced cellular proliferation assay indicate that at least two of the cell surface molecules examined (CD45RB and LFA-1) deliver similar costimulatory signals to TH₀, TH₁, or TH₂ cells. Furthermore, inhibition of the antigen-specific proliferative responses of the three T-cell clones, examined by antibodies binding CD45RO and CD45RB, indicates that interaction of CD45RO and CD45RB with their B-cell ligand plays an important role in CD4⁺ T-cell activation. It is possible that the inhibition could result from anti-CD45 antibody binding, generating a signal that inhibits T-cell proliferation. However, the proliferative responses generated by cross-linking CD45RB and CD3 indicate that CD45RB recognition of its counterstructure generates a costimulatory signal required for CD4⁺ T-cell proliferation. The weak or absent costimulatory signal generated when VLA-4, CD45RO, or CD45RA was cross-linked with CD3 may be due to the fact that these antibodies did not bind to activating epitopes. Similarly, the failure of antibodies, binding to LFA-1, VLA-4, and CD45RA, to inhibit the antigen-specific proliferative responses of the three T-cell clones examined may also be due to the fact that these antibodies recognize epitopes that do not block counterligand binding. The mechanism or mechanisms by which isoforms of CD45 differentially provide costimulatory signals to T cells is unknown at present. The solid-phase antibody-induced proliferation assay results are consistent with the possibility that isoform-specific interactions of the extracellular do-

main of CD45 with other cell surface antigens^{10, 17} indirectly regulate the intracellular signals generated when a CD45 isoform is triggered.¹⁶ It is also possible that the anti-CD45 antibodies used in this study differ in their tendency to prevent or facilitate interaction of CD45 with other molecules and that these differences are unrelated to the specific isoform recognized by each antibody. Alternatively, isoform-specific differences in the tyrosine phosphatase activity of the cytoplasmic domain of CD45 could also influence the ability of a CD45 isoform to provide a costimulatory signal to T cells.

A previous analysis of CD45-deficient mutants of a human leukemic T-cell line indicated that CD45 expression is essential for T-cell activation through the T-cell antigen receptor/CD3 complex,³² yet other investigators noted that CD45 could deliver activating or inhibitory costimulatory signals to human lymphocytes.^{16, 33} Our findings differ from those of others because we have characterized the response of cloned human T cells, rather than freshly isolated populations of polyclonal lymphocytes. None of the T-cell clones proliferated when stimulated by anti-CD3 antibody alone. However, anti-CD3 antibody stimulated the proliferation of highly purified CD4⁺ T cells isolated from human peripheral blood in control experiments (data not shown). Antigen-primed and resting CD4⁺ human T cells have been shown to differ in the costimulatory molecules preferentially triggering proliferative responses.³⁴ The use of T-cell clones also eliminates potential artifacts introduced by contaminating non-T cells, as well as heterogeneity in the proliferative response caused by variation between donors or from interactions between different subsets of CD4⁺ T cells. The T-cell clones used in this study will be useful in future studies to determine whether there are other properties, differentially expressed genes, or intracellular signalling events that distinguish human CD4⁺ T cell subsets. It also remains to be determined whether there is a developmental stage in which activation of a particular costimulatory molecule on the surface of a CD4⁺ T cell influences the profile of cytokines that a T cell produces when subsequently activated.

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