Human CD4 Helper T Cell Activation: Functional Involvement of Two Distinct Collagen Receptors, 1F7 and VLA Integrin Family
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Summary
In the present study, we showed that activation of human CD4 T cells can be induced by anti-CD3 and collagen in a serum-free system. This activation was inhibited by the addition of peptides containing the RGD or Gly-Pro-X sequences. Significantly, we demonstrated that both the 1F7 (CD26) structure and the VLA integrin family, particularly the VLA 3 complex, contribute to the functional interaction between collagen and CD4 cells since anti-1F7 and anti-VLA-3 specifically inhibited this collagen-induced CD4 cell activation. Biochemical studies showed that the 1F7 structure is not a member of the VLA integrin family. These results thus indicated that two different families of antigens serve as functional collagen receptors for CD4 T cell activation.

Materials and Methods

Cell and Reagents. Highly purified human CD4 T cells were obtained as described previously (8, 9). Collagen I, III, and IV, and BSA were from Sigma Chemical Co., St. Louis, MO. Laminin was from Collaborative Research, Bedford, MA. Antibodies reactive against T cell surface antigens used in this paper were described previously (7-9). Anti-VLA-2 (12F1) was obtained from Drs. K. Pischel and V. Woods, University of California, San Diego; anti-UCHL1 was from Dr. P. Beverley, Imperial Cancer Research Fund, United Kingdom; anti-VLA-5 (mAb 16) from Dr. K. Yamada, National Institutes of Health, Bethesda, MD. Anti-VLA-3 (J43), a gift from Dr. A. Albino, Memorial Sloan-Kettering Cancer Center (New York, NY), was in ascites form and was extensively dialyzed against PBS before experimentation. Peptides containing the Gly-Pro-X sequences were from Sigma Chemical Co. RGD-containing peptides were from Telios Pharmaceuticals, San Diego, CA.

Proliferation Assays. Proliferation assays were done as described earlier (9). For inhibition studies, cells were incubated with all tested antibodies or peptides for 30 min at 37°C before culture and then placed in 96-well plates for proliferation assay. Data are expressed as mean of triplicate samples and are representative of three separate experiments. Each standard error was <15%. [3H]Thymidine incorporation for cells incubated with collagen alone was <500 cpm.

Biochemical Analyses. For Fig. 3 a, T lymphocytes were stimulated with PHA (0.25 μg/ml) and rIL-2 (40 U/ml) (Biogen, Cambridge, MA) for 10 d, while for Fig. 3 b, resting CD4 cells were used after being prepared as described earlier. Biochemical analyses were then done as described previously (7, 9). All samples were analyzed under reducing conditions.

Results and Discussion

Inhibition of Anti-CD3 plus Collagen-induced Human CD4 T Cell Activation by Anti-1F7 and Anti-4B4 (CD29) mAb. As shown in Fig. 1 a, when combined with anti-CD3 antibody (0.1 μg/ml), collagen types I, III, and IV could elicit CD4 cell proliferation, with collagen type I being most synergistic, while anti-CD3 or collagen alone cannot elicit such activation. We then examined whether the interaction of collagen with an integrin receptor or other class(es) of molecule on...
CD4 cells was required for the observed cell activation. Since anti-4B4 (CD29) antibody (10) reacts with the common $\beta$ subunit associated with several $\alpha$ subunits of the VLA integrin family, we undertook experiments to determine whether anti-4B4 and antibodies recognizing other surface structures could modulate the activation of CD4 cells. In Fig. 1b, addition of anti-4B4 as well as anti-1F7, but not control antibodies, at culture initiation markedly inhibited proliferation of CD4 cells incubated in a serum-free culture system in the presence of anti-CD3 (0.1 $\mu$g/ml) and type I collagen (2 $\mu$g/ml). Inhibition of anti-CD3 plus collagen-induced proliferation by anti-VLA-3 but not anti-VLA-2. Addition of anti-VLA-3 but not anti-VLA-2 antibodies at culture initiation inhibited proliferation of CD4 cells incubated in a serum-free culture system in the presence of anti-CD3 (0.1 $\mu$g/ml) and type I collagen (2 $\mu$g/ml).

Inhibition of Anti-CD3 plus Collagen-induced CD4 T Cell Activation by Peptides Containing RGD or Gly-Pro-X Sequences. Many of the integrin receptors recognize sites that include the tripeptide Arg-Gly-Asp (RGD), a sequence that is common to many extracellular matrix proteins, including collagen, and which is thought to play a key role in cell adhesion and spreading (2, 13, 14). As shown in Fig. 2a, when the hexapeptide Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) containing the RGD sequence was added to cell cultures, proliferation of CD4 cells in response to anti-CD3 plus type I collagen was inhibited in a dose-dependent fashion. In contrast, no inhibition was observed with the related control hexapeptides GRGESP and GRADSP. The central structure of collagen can be regarded as being composed of repeating tripeptides which is thought to play a key role in cell adhesion and spreading (2, 13, 14). As shown in Fig. 2a, when the hexapeptide Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) containing the RGD sequence was added to cell cultures, proliferation of CD4 cells in response to anti-CD3 plus type I collagen was inhibited in a dose-dependent fashion. In contrast, no inhibition was observed with the related control hexapeptides GRGESP and GRADSP.

CD4 T cells is inhibitable by antibodies recognizing 1F7 and the VLA family of integrin receptors. (a) Collagen can induce proliferation of CD4 cells in combination with anti-CD3 (0.1 $\mu$g/ml). When purified CD4 cells were cultured with anti-CD3 antibody alone in serum-free media, there was no proliferation above background, using antibody concentration of up to 5 $\mu$g/ml. (b) Inhibition of anti-CD3 plus collagen-induced proliferation by anti-1F7 and anti-4B4. Addition of anti-1F7 or anti-4B4 at culture initiation inhibited proliferation of CD4 cells incubated in a serum-free culture system in the presence of anti-CD3 (0.1 $\mu$g/ml) and type I collagen (2 $\mu$g/ml). (c) Inhibition of anti-CD3 plus collagen-induced proliferation by anti-VLA-3 but not anti-VLA-2. Addition of anti-VLA-3 but not anti-VLA-2 antibodies at culture initiation inhibited proliferation of CD4 cells incubated in a serum-free culture system in the presence of anti-CD3 (0.1 $\mu$g/ml) and type I collagen (2 $\mu$g/ml).

Figure 1. Anti-CD3 plus collagen-induced activation of human CD4 T cells is inhibitable by antibodies recognizing 1F7 and the VLA family of integrin receptors. (a) Collagen can induce proliferation of CD4 cells in combination with anti-CD3 (0.1 $\mu$g/ml). When purified CD4 cells were cultured with anti-CD3 antibody alone in serum-free media, there was no proliferation above background, using antibody concentration of up to 5 $\mu$g/ml. (b) Inhibition of anti-CD3 plus collagen-induced proliferation by anti-1F7 and anti-4B4. Addition of anti-1F7 or anti-4B4 at culture initiation inhibited proliferation of CD4 cells incubated in a serum-free culture system in the presence of anti-CD3 (0.1 $\mu$g/ml) and type I collagen (2 $\mu$g/ml). (c) Inhibition of anti-CD3 plus collagen-induced proliferation by anti-VLA-3 but not anti-VLA-2. Addition of anti-VLA-3 but not anti-VLA-2 antibodies at culture initiation inhibited proliferation of CD4 cells incubated in a serum-free culture system in the presence of anti-CD3 (0.1 $\mu$g/ml) and type I collagen (2 $\mu$g/ml).

Figure 2. Inhibition of anti-CD3 plus collagen-induced proliferation of CD4 T cells by peptides containing RGD or Gly-Pro-X sequences. (a) Proliferation of CD4 T cells cultured in anti-CD3 (0.1 $\mu$g/ml) plus type I collagen (2 $\mu$g/ml)-coated wells in a serum-free system was inhibited by GRGDSP but not GRADSP or GRGESP peptides. (b) Proliferation of CD4 T cells cultured in similar conditions as a was inhibited by Gly-Pro-Gly-Gly, Gly-Pro-Ala, and Gly-Pro-OH-Pro but not Gly-Gly-Gly and Gly-Gly-Val peptides.
tion of GlyProAla, GlyProGlyGly, and GlyProOHPro, but not GlyGlyGly and GlyGlyVal. Significantly, the addition of peptides containing the GlyProX sequences does not inhibit CD4 cell activation in the presence of fibronectin and anti-CD3, while the addition of peptides containing the RGD sequence does not inhibit CD4 cell proliferation induced by anti-CD3 plus PMA or anti-CD3 plus IL-2 (data not shown). Together, the above results suggested that peptides that include the RGD and GlyProX sequences contain important sites for the interaction of collagen with CD4 lymphocytes.

The 1F7 Molecule and VLA Integrin Family Belong to Distinct Families of Structures. In view of the inhibitory effects on cell–collagen interaction exhibited by antibodies recognizing the 1F7 structure and the VLA integrin family, structural comparison of 1F7 and the VLA integrin family was done by SDS-PAGE. From lysates of PHA-stimulated T cells, anti-1F7 antibody precipitated the diffuse single band of the 110-kD glycoprotein, and anti-4B4 precipitated the diffuse band of 130–135-, the 165-, and 185-kD glycoproteins, as previously described (7, 9). Anti-VLA-2 and anti-VLA-3 antibodies precipitated the 130- plus 165-kD and the 130- plus 135-kD glycoproteins, respectively (Fig. 3 a). Since it is still possible that cell surface structures may be altered after T cell activation, we then performed sequential immunoprecipitation using lysates from resting CD4 T cells to exclude this possibility. Data from these studies showed that there was no crossreaction between anti-1F7 and anti-4B4 antibodies (Fig. 3 b). The above results thus indicated that the 1F7 molecule and VLA integrin family belong to distinct families of structures.

In this paper we thus demonstrated that the 1F7 molecule and the VLA integrin family are functional collagen receptors on human CD4 cells. Anti-1F7, as well as anti-4B4, also prevented the adhesion and spreading of mesothelioma cells on plastic wells coated with collagen (data not shown). It is important to note that anti-1F7 recognizes the CD26 antigen, which includes the structure recognized by anti-Tat and anti-DPPIV antibodies. Recently, Hanski et al. demonstrated that rat liver DPPIV has a binding affinity for collagen (6), while Bauvois reported that mouse fibroblast DPPIV may be a collagen-binding protein (5). While further studies are required to determine whether 1F7 and VLA-3 are two independent collagen receptors on human CD4 T cells or whether either molecule serves as an accessory molecule that facilitates collagen binding to its receptor, the identification of the 1F7 antigen as a functional collagen receptor, one distinct from the VLA integrin family, suggests the existence of distinct classes of functional collagen receptors that mediate collagen-dependent T cell function.

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