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Tenascin, an extracellular matrix protein, exerts immunomodulatory activities

(cell adhesion/immunosuppression/T-cell activation/antigen presentation)

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ABSTRACT **Tenascin is a nonubiquitous extracellular matrix protein mainly expressed during morphogenesis in embryonal life. In adults it reappears in malignant tumors and during inflammation and tissue repair. Extracellular matrix proteins can alter cell morphology, adhesion, motility, differentiation, and growth. Since cells of the immune system can express receptors for extracellular matrix, we investigated the effects of tenascin on human monocytes and T and B lymphocytes. Tenascin inhibited monocyte adhesion to fibronectin and enhanced the LFA-1 (lymphocyte function-associated antigen 1)-dependent clustering of Epstein–Barr virus-transformed B cells. The physiological consequences of the effects of tenascin were studied in several T-cell activation models. Tenascin inhibited T-cell activation induced by a soluble antigen (tetanus toxoid), alloantigens, or the mitogen concanavalin A. However, T-cell activation with phytohemagglutinin, crosslinked anti-CD3 antibody, or a mixture of ionomycin and phorbol ester was not inhibited by tenascin. Tenascin did not prevent interleukin 2-dependent T-cell growth or the cytolytic activity of an antigen-specific CD4$^+$ T-cell clone. These results suggest that tenascin alters the adhesion properties of human monocytes, B cells, and T cells. The *in vitro* immunosuppressive activity of tenascin might be due to abrogation of an accessory cell function at an early stage of the interaction between antigen-presenting cells and T cells.**

Physiological, antigen-specific T-cell activation, is initiated by the T-cell receptor recognizing antigen fragments bound to a major histocompatibility complex (MHC)-encoded molecule on the surface of an antigen-presenting cell (1). In addition, many other accessory molecules are required for successful T-cell activation, proliferation, and target cell killing (2). Recently, several adhesion receptor–ligand interactions have been described—including the interactions of lymphocyte function-associated antigen 1 (LFA-1) with intercellular adhesion molecule 1 (ICAM-1) (3), CD2 with LFA-3 (4), CD4 with MHC-class II molecules (5), and CD8 with MHC class I molecules (6)—and their role in T-cell function has been thoroughly investigated (reviewed in refs. 7 and 8). In addition to cellular adhesion receptors, cells of the immune system, including T cells, express receptors for extracellular matrix (ECM) proteins such as the very late antigen (9) proteins of the integrin Arg-Gly-Asp (RGD)-receptor superfamily (10). This fact has suggested a potential physical and functional interaction between ECM proteins and lymphoid or myeloid cells (11). From other cellular systems it is well known that ECM proteins can profoundly alter cellular morphology, adhesion, motility, growth, and differentiation (reviewed in refs. 12–14). Apart from a few studies (15–17) the potential effects of ECM proteins on the cells of the immune system have not been studied.

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Here we investigate the effects of the ECM protein tenascin on cells of the immune system. Tenascin is a disulfide-linked hexameric glycoprotein (190–240 kDa per subunit) originally described as myotendinous antigen (18, 19) and also discovered independently by others (20–23). A striking feature of tenascin is its limited spatiotemporal distribution, mainly restricted to embryonal tissues undergoing morphogenesis such as muscles and tendons, mammary glands, hair follicles, teeth, kidney, bone, and cartilage (18, 24–26). In adult life it reappears in the stroma of malignant tumors (27) and in wounds (28). Recently it has been demonstrated that tenascin inhibits integrin-dependent attachment of chicken embryo fibroblasts to fibronectin, laminin, and the Gly-Arg-Gly-Asp-Ser (GRGDS) peptide (29). Because the expression of tenascin is in some organs restricted to embryogenesis, inflammation, and epithelial malignancies, it was thought that tenascin may have a regulatory role in epithelial–mesenchymal cell interactions (27).

Results presented here demonstrate that tenascin interacts with human monocytes, T cells, and Epstein–Barr virus-transformed B (EBV-B) cells and alters their adhesive properties. Furthermore, tenascin inhibits T-cell activation mediated by some but not all activators.

MATERIALS AND METHODS

ECM Proteins and Monoclonal Antibodies (mAbs). Tenascin was purified from primary chicken embryo fibroblast cultures by monoclonal anti-tenascin immunoaffinity chromatography (24). The purity of the preparations were >90% as checked by SDS/PAGE. Fibronectin was isolated from horse serum by affinity chromatography on gelatin-Sepharose (30). Fibronectin-free fetal bovine serum was obtained by absorption of fibronectin on gelatin-Sepharose. mAbs were obtained from the following sources: anti-LFA-1 (25.3.1, IgG1) from Immunotech (Marseille); anti-CD3 (OKT3, IgG2a) and anti-CD2 (OKT11, IgG2a) from Ortho Diagnostics; anti-Leu-M5 (IgG2b), anti-Leu-M3 (IgG2a), anti-Leu-16 (IgG1), anti-Leu-4 (IgG1), and anti-HLA-DR (IgG2a) from Becton Dickinson; anti-MHC class I (W6/32, IgG2a) from American Type Culture Collection. Fluorescein-conjugated goat anti-mouse immunoglobulin was from Becton Dickinson.

Blood Donors, Cell Purification, and Cell Lines. From a pool of healthy donors, three high responders to the recall antigen tetanus toxoid, one high responder to the purified protein derivative of tuberculin (PPD), and several pairs of allogeneic

Abbreviations: EBV-B cell, Epstein–Barr virus-transformed B cell; ECM, extracellular matrix; ICAM-1, intercellular adhesion molecule 1; IL-2, interleukin 2; LFA-1, lymphocyte function-associated antigen 1; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate; PPD, purified protein derivative of tuberculin; TT-C, tetanus toxoid C fragment.

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responders were selected. Peripheral blood mononuclear cells (PBMCs) were isolated from blood by the standard Ficoll-Paque procedure (Pharmacia) and resuspended in complete culture medium [RPMI 1640 (Amimed, Muttenz, Switzerland) supplemented with 15% fetal bovine serum (GIBCO), 1 mM sodium pyruvate (Seromed, Biochrom KG, F.R.G.), 50 μM 2-mercaptoethanol (Fluka), 100 units of penicillin and 100 μg of streptomycin per ml, and 2 mM L-glutamine (Amimed)]. T cells were purified from the PBMCs by using nylon wool columns (31). After two column purifications the cells obtained were >96% Leu-4$^+$ (CD3$^+$) (T cells), <0.5% Leu-M3$^+$ (monocytes), and <0.5% Leu-16$^+$ (B cells) as assessed by microfluorescence (Becton Dickinson manual) and were used as purified T cells. Monocytes were purified from PBMCs first by T-cell depletion through rosette formation with sheep erythrocytes (32) and second, by density separation in a discontinous Percoll gradient (Pharmacia) (33). The cells collected at the medium–1.062 g/ml density interface were ≥95% Leu-M3$^+$, 2% Leu-4$^+$, and 2% Leu-16$^+$ and were used as purified monocytes.

EBV-B cell lines were established from PBMCs. PBMCs (2.5×10^5 per ml) were mixed with an equal volume of the supernatant from the B 95-8 cell line and supplemented with cyclosporin A (0.6 μg/ml; Sandoz, Basel). Cells were seeded (1 ml per well) into 48 wells of a microtiter plate (Falcon 3072), and fed once a week until clusters of transformed cell growth were seen (2–3 weeks).

PPD-specific T-cell clones were established according to ref. 34. PBMCs (10^6) from a PPD-positive donor were plated in a 2-ml culture well (Costar), with PPD (25 μg/ml; Statens Serum Institute, Copenhagen). At day 4 recombinant human interleukin 2, (IL-2, 50 units/ml; Sandoz, Vienna) was added. Two weeks later T-cell blasts were restimulated using freshly isolated syngeneic PBMCs preincubated with PPD and irradiated (2500 rads; 1 rad = 0.01 Gy); 2×10^5 T-cell blasts and 10^6 PBMCs were incubated per 2-ml well. Growing cells were cloned by limiting dilution (0.3 cell per well) and the clones were tested for antigen (PPD) specificity in a standard proliferation assay. One clone, N411, was used in this study.

T-Cell Activation Assays. The assays were performed in 96-well microculture plates (Falcon) in 200-μl volumes. For antigen-specific activation, monocytes (3×10^5 per ml) were incubated at 37°C for 16 hr with tetanus toxoid C fragment protein (TT-C, 25 μg/ml, molecular mass 47 kDa; Calbiochem) in a polypropylene tube (Costar), irradiated (2000 rads), washed, and added to purified T cells (3×10^4 monocytes plus 10^5 T cells per well). For alloactivation in a two-way mixed lymphocyte reaction, the cells from two donors were mixed at 1.5×10^5 cells per donor per well for 3 days. For Con A, anti-CD3 mAb, or phytohemagglutinin (PHA) activation, PBMCs (10^5 per well) were plated in the presence of Con A (5 μg/ml; Pharmacia), OKT3 mAb (250 ng/ml), or PHA (PHA-L, 3 μg/ml; Sigma). For activation with ionomycin and phorbol 12-myristate 13-acetate (PMA), purified T cells (5×10^4 per well were incubated with ionomycin (250 ng/ml; Calbiochem) and PMA (10 ng/ml; Sigma). For IL-2-dependent T-cell proliferation, T cells, 10 days after PHA activation and expansion in medium containing IL-2, were seeded at 10^5 cells per well in the presence of IL-2 (100 units/ml).

Response Measurement. [^{3}H]Thymidine incorporation was used as the parameter for T-cell activation. [Methyl-^{3}H]Thymidine (1 μCi per well, 2.0 Ci/mmol; Amersham; 1 Ci = 37 GBq) was added for 16 hr at different time points (i.e., at 48 hr for IL-2-dependent growth or for Con A, anti-CD3 mAb, or PHA activation; 96 hr for allogeneic activation; 120 hr for antigen-mediated activation). Cultures were harvested on glass fiber filters (PHD 200A cell harvester, Cambridge Technology, Cambridge, MA), and the incorporated radioactivity was measured by liquid scintillation counting (Packard). Each data point represents the mean of triplicate cultures. Results are expressed as relative T-cell activation based on the positive (100%) and negative (0%) control conditions. Standard deviation (SD) and number of experiments (n) are given.

Cytotoxicity Assay. Killing was assessed by ^{51}Cr release according to ref. 35, using the CD4$^+$CD8$^-$, MHC class II-restricted, PPD-specific T-cell clone N4-11 (unpublished work) as effector cell and the syngeneic EBV-B cell line (N4-E) preincubated with PPD (50 μg/ml) as target cell. EBV-B without PPD preincubation served as negative control. Specific killing was calculated from the radioactivity released after 4 hr as follows: % specific killing = [(cpm experimental − cpm spontaneous)/(cpm maximal − cpm spontaneous)] × 100.

Cell Adhesion Assays. ELISA plates (Dynatech) were coated with 1% bovine serum albumin (Fluka) or with tenascin or fibronectin (10 μg/ml) in phosphate-buffered saline for 2 hr at 37°C and then washed and blocked with RPMI 1640 medium containing 7% fibronectin-free fetal bovine serum for 30 min at room temperature. Cells (10^7 in 0.2 ml) were labeled with $Na_2{}^{51}CrO_4$ (100 μCi; Amersham) for 1 hr at 37°C, washed with medium, and plated (2×10^6 cells per well) under the indicated conditions. Plates were centrifuged at 25 × g for 2 min and incubated at 37°C. After 1 hr, 50% of a control supernatant was collected to determine the spontaneous release of radioactivity. Unbound cells were removed by washing the wells with medium five times. Attached cells were lysed with 1% (vol/vol) Nonidet P-40/0.1 M NaOH and released radioactivity was measured with a γ counter. Percent binding was calculated as follows: % binding = [cpm from bound cells/(cpm input − cpm spontaneous)] × 100.

Cell Clustering Assay. For these experiments EBV-B cells, freshly purified T cells, and PHA-activated T-cell blasts (restimulated >7 days) were used. The cells were washed with phosphate-buffered saline and resuspended by repeated pipetting in complete medium with 7% fetal bovine serum to obtain a single-cell suspension. Experiments were performed in 96-well microtiter plates (2×10^5 cells per well, 100-μl final volume). mAbs [anti-LFA-1 (25.3.1), anti-CD2 (OKT11), or anti-MHC class I (W6/32)], tenascin, and fibronectin were used at indicated concentrations. Micrographs were taken after 3 hr through an inverted microscope.

RESULTS

Monocytes, But Not T Cells or EBV-B Cells, Adhere to Tenascin and Tenascin Inhibits Monocyte Attachment to Fibronectin. To investigate the ability of tenascin to interact with cells of the immune system, adherence of monocytes, T cells, and EBV-B cells to tenascin was investigated. Only monocytes adhered to tenascin (Fig. 1*A*). Soluble fibronectin and tenascin were able to block the attachment of monocytes to immobilized fibronectin by 50% and 40%, respectively (Fig. 1*B*).

Tenascin Induces Aggregation of EBV-B Cells. EBV-B cells, though not adherent, are able to undergo spontaneous aggregation in suspension. This kind of aggregation has been shown to be largely LFA-1/ICAM-1-dependent (36). Addition of tenascin to monodisperse EBV-B-cell suspensions (obtained by vigorous pipetting) markedly enhanced cluster formation (Fig. 2 *a–d*). Fibronectin did not show this effect (data not shown). The clustering effect was observed even when tenascin was coated on the bottom of the well (Fig. 2*e*). The effect of soluble tenascin (Fig. 2*g*) was inhibited by anti-LFA-1 mAb (Fig. 2*h*). The control antibody, anti-HLA-DR, was ineffective (data not shown). On the other hand, T-cell blasts quickly underwent cluster formation when tenascin and anti-LFA-1 mAb (but not anti-CD3, anti-CD2, or anti-MHC class I) were both present, whereas either

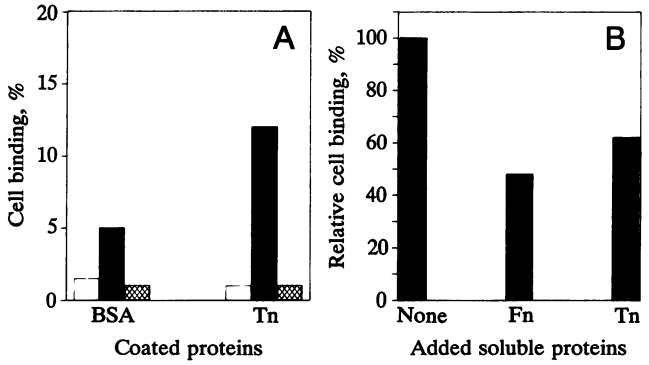

FIG. 1. Cell attachment to tenascin and inhibition by tenascin of monocyte adhesion on fibronectin. (*A*) ^{51}Cr-labeled monocytes (solid bars), EBV-B cells (cross-hatched bars), or T cells (open bars) were incubated at 37°C in wells (2×10^5 cells per well) precoated with bovine serum albumin (BSA) or tenascin (Tn). The proportion of attached cells was determined as described in *Materials and Methods*. (*B*) The ability of soluble tenascin (Tn) or fibronectin (Fn) to counteract the attachment of monocytes to immobilized fibronectin was tested by addition of the proteins (10 μg/ml) to 2×10^5 ^{51}Cr-labeled monocytes incubated for 1 hr at 37°C in fibronectin-coated wells. The experiment was performed in the presence of 7% fetal bovine serum (fibronectin-free). One hundred percent relative binding was the unaffected monocyte binding on fibronectin (50% specific cell binding). In another experiment the addition of BSA (50 μg/ml) as negative control had no effect on cell attachment (data not shown).

tenascin or anti-LFA-1 mAb alone was ineffective (data not shown).

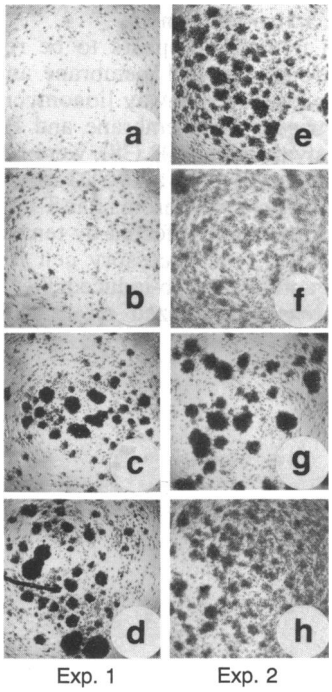

FIG. 2. Tenascin enhances the homotypic aggregation of EBV-B cells. The effect of tenascin on EBV-B lymphocytes was tested under different conditions. In experiment 1, 2×10^5 EBV-B cells were incubated alone (negative control) (*a*) or in the presence of tenascin at 5, 10, or 40 μg/ml (*b–d*, respectively). A similar effect was induced by immobilized tenascin as shown in experiment 2. EBV-B cells clustered in wells precoated with tenascin at 10 μg/ml (*e*) as compared to cells in wells precoated with bovine serum albumin (*f*). The aggregation induced by the addition of tenascin at 10 μg/ml (*g*) was strongly reversed by the addition of anti-LFA-1 mAb at 10 μg/ml (*h*).

Tenascin Inhibits Some But Not All Pathways of T-Cell Activation. Possible effects of tenascin on T-cell activation were investigated using four different assays: (*i*) TT-C-induced T-cell activation, to assay for antigen-specific T-cell responses; (*ii*) mixed lymphocyte reaction, to assay for allogeneic T-cell activation; (*iii*) polyclonal activation induced by anti-CD3 mAb, PHA, or Con A; (*iv*) ionomycin/PMA-induced T-cell activation. All experiments were performed with fresh PBMCs. Tenascin inhibited allogeneic and TT-C-specific T-cell activation by as much as 90% (Fig. 3 *A* and *B*) and Con A-mediated T-cell activation by as much as 60% (Fig. 3*C*) but did not affect PHA, anti-CD3 mAb, or ionomycin/PMA T-cell activation (Fig. 3 *D–F*). Stimulation of T cells with a suboptimal concentration of PHA or anti-CD3 mAb was not inhibited by tenascin (data not shown). The tenascin effects were dose-dependent and reproducible for all eight donors tested. In contrast fibronectin, used as a control, had no effect in any of the assays (Fig. 4 and data not shown). Because of the LFA-1-related effects found in the EBV-B and T-cell clustering experiments, we compared tenascin effects with those obtained by the addition of anti-LFA-1 mAb in the different T-cell activation assays. As expected, anti-LFA-1 mAb inhibited antigen-specific, allo-

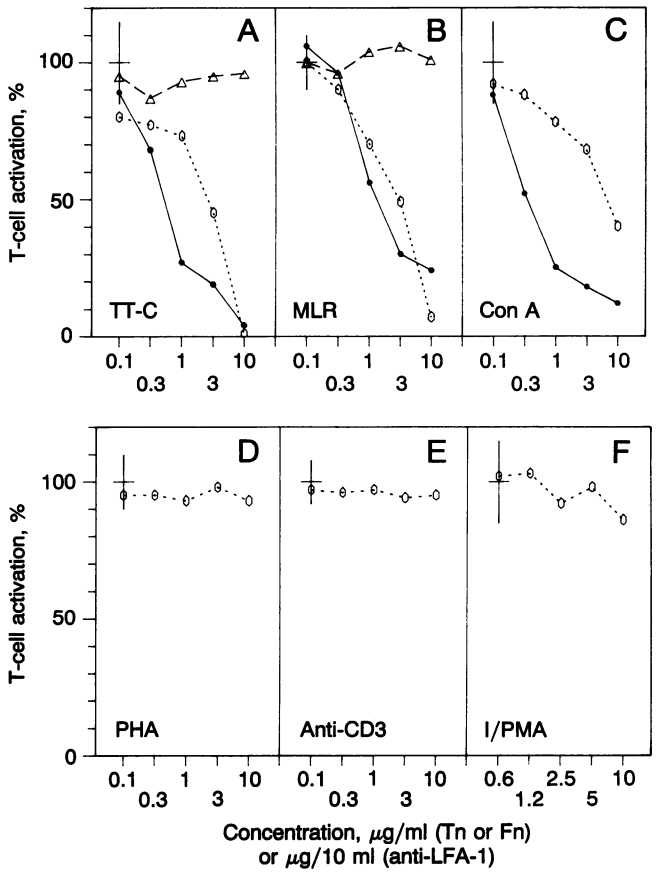

FIG. 3. Effects of tenascin on T-cell activation induced by different stimuli. Tenascin (Tn, ○), fibronectin (Fn, △), or anti-LFA-1 mAb (●) was added to six different T-cell activation assays at the indicated final concentrations. Tenascin inhibited activation mediated by TT-C (*A*), mixed lymphocyte reaction (MLR) (*B*), or Con A (*C*) but not activation induced by PHA (*D*), anti-CD3 mAb (*E*), or ionomycin plus PMA (I/PMA) (*F*). Fibronectin had no inhibitory activity (*A*, *B*, and data not shown), whereas anti-LFA-1 mAb inhibited TT-C, allogeneic, Con A, and anti-CD3 activation (*A–C* and data not shown). [^{3}H]Thymidine incorporation in stimulated control cultures (taken as 100%) was as follows: 25,866 cpm, SD < 15%, $n = 5$ (*A*); 16,717 cpm, SD ≤ 10%, $n = 2$ (*B*); 41,167 cpm, SD ≤ 15%, $n = 3$ (*C*); 121,320 cpm, SD < 10%, $n = 3$ (*D*); 106,570 cpm, SD < 10%, $n = 4$ (*E*); 52,562 cpm, SD < 10%, $n = 2$ (*F*).

geneic, and Con A- (Fig. 3 *A–C*) or anti-CD3-mediated activation but did not affect PHA or ionomycin/PMA activation (data not shown).

The Tenascin Effect Is Restricted to the Time of Antigen-Presenting Cell–T-Cell Contact and Is Reversed by Stronger Activation Signals. To better understand the inhibitory activity of tenascin, we investigated its effect on the kinetics of T-cell activation in the antigen-specific activation assay (Fig. 4*A*). Tenascin was inhibitory only when added early in the culture period—i.e., up to day 2 (80% inhibition). With later addition this inhibitory effect gradually declined. Addition of fibronectin had no effect. The kinetics of anti-LFA-1-mediated inhibition were similar to those of the tenascin effect, although the effectiveness started decreasing after the first 24 hr (data not shown). Preincubation of either monocytes or T cells with tenascin and washing before mixing had no inhibitory effect on the proliferative response of T cells (Fig. 4*B*). In contrast, readdition of tenascin at the start of culture restored its suppressive activity. Tenascin had no inhibitory activity on IL-2-dependent T-cell growth (Fig. 4*C*). To further characterize the quality of the inhibitory activity of tenascin, we titrated both the TT-C antigen and tenascin (Fig. 4*D*). The tenascin-mediated inhibition of T-cell activation could be partially reversed by increasing the intensity of the activation signal (i.e., by increasing the TT-C antigen concentration).

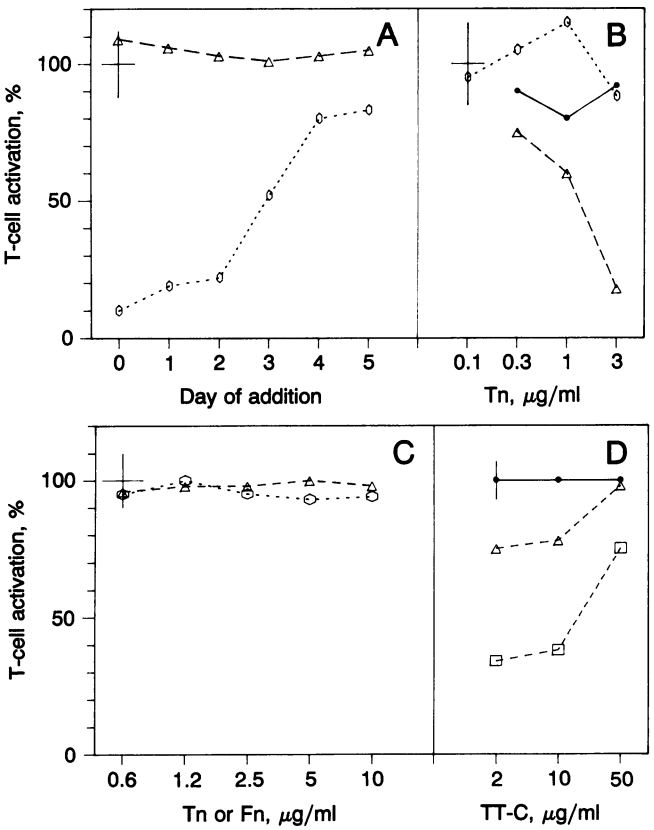

FIG. 4. Kinetics of the inhibition of T-cell activation by tenascin. (*A*) Kinetics of tenascin effects. Tenascin (○) or fibronectin (△) was added from day 0 to day 5 to an ongoing TT-C activation assay. (Stimulated control cultures: 21,080 cpm, SD ≤ 15%, n = 2.) (*B*) Effects of preincubation of cells with tenascin (Tn). Monocytes (○) or T cells (●) were preincubated alone (control cultures) or in the presence of tenascin at the indicated concentrations for 16 hr, washed, and then combined for the proliferation assay. As positive control (△) tenascin was added into the culture at the start. (Stimulated control cultures: 26,877 cpm, SD ≤ 15%, n = 1.) (*C*) Effects of tenascin on proliferating T cells. Tenascin (Tn, ○) or fibronectin (Fn, △) was added to T-cell blasts growing in the presence of IL-2. (Stimulated control cultures; 64,450 cpm for T cells growing in the presence of IL-2 and 435 cpm for cultures without IL-2, SD < 10%, n = 2.) (*D*) Effect of antigen concentration on tenascin inhibition. T-cells were activated with TT-C at 2, 10, or 50 μg/ml in the absence of tenascin or in the presence of tenascin at 1 μg/ml (△) or 10 μg/ml (□). [Stimulated control cultures: 4808 cpm; 19,153 cpm; 65,168 cpm (for TT-C at 2, 10, and 50 μg/ml, respectively), SD < 15%, n = 2.]

Tenascin Does Not Block Interaction Between T-Cell Receptor and Antigen–MHC Protein Complex or Between LFA-1 and ICAM-1. To exclude a possible inhibition of recognition of the antigen–MHC protein complex by the T-cell receptor, we tested tenascin effects in an antigen-specific, MHC class II-restricted target-cell killing model that is known to require the same α/β T-cell receptor involvement as in the proliferative response (37). The lack of inhibition (Fig. 5) indicates that tenascin did not simply block the binding of T-cell receptor to antigen–MHC protein complex or the interaction of LFA-1 with ICAM-1. As expected, anti-LFA-1 mAb almost completely inhibited the killing activity.

DISCUSSION

In this study we investigated the effects of the ECM protein tenascin on cells of the immune system and found two main effects: (*i*) tenascin altered the adhesion properties of monocytes, EBV-B cells, and T cells; (*ii*) tenascin inhibited T-cell activation mediated by soluble antigen, alloantigens, or Con A. Activation induced by PHA, anti-CD3 mAb, or ionomycin/PMA were not affected. Optimal inhibition of T-cell activation was obtained when tenascin was added at the start of the culture; preincubation of the cells or later addition had little or no effect. The inhibitory effect was not due to inhibition recognition of the antigen–MHC complex by the T-cell receptor and could be reversed by increasing the intensity of the activation signals.

The tenascin inhibition appears to be related to signal transduction through the cell membrane and to the T-cell receptor/CD3 activation pathway [ionomycin/PMA activation, which bypasses the membrane and PHA activation, which acts mainly through CD2 (38), were both unaffected]. The finding that Con A- and anti-CD3 mAb-induced T-cell activation, both of which act through the T-cell receptor–CD3 complex, are differentially affected by tenascin may appear surprising. However, the accessory cell requirements for Con A- and for anti-CD3 mAb-induced T-cell activation are different. Whereas immobilized or crosslinked anti-CD3

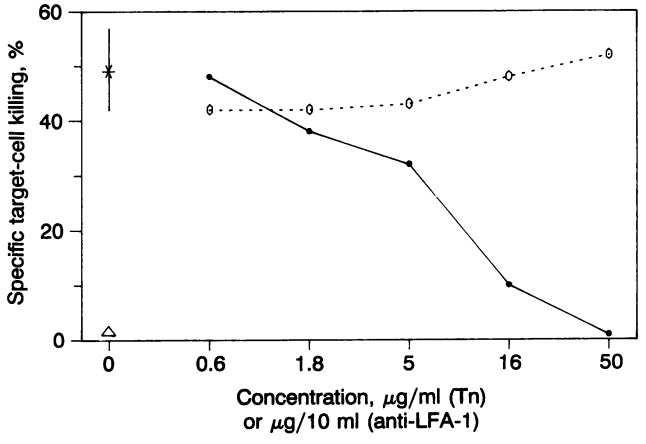

FIG. 5. Tenascin does not block antigen-specific target-cell killing. Tenascin (Tn, ○) or anti-LFA-1 mAb (●) was added at the indicated final concentration to a PPD-specific target-cell killing assay. PPD-preincubated EBV-B target cells (N4-E) and effector T cells (N4-11) were mixed at a 1:20 ratio. In this 4-hr ^{51}Cr-release assay, maximal release was 866 cpm and spontaneous release was 274 cpm (SD ≤ 8%, n = 2).

mAb can activate T cells without accessory cells, Con A activation is strictly accessory cell-dependent (38, 39).

The lack of inhibition of the T-cell killing function by tenascin clearly indicates that tenascin does not block T-cell receptor function or LFA-1/ICAM-1 interactions directly. On the other hand the EBV-B-cell clustering experiment suggests that the LFA-1 molecule might be involved in some of the tenascin effects. It is possible that tenascin blocks some accessory functions during the contact between antigen-presenting cells and T cells that is necessary for successful physiological T-cell activation.

A physical interaction of tenascin with LFA-1 at a binding site other than the ICAM-1 binding site, giving an inhibitory signal to the cell, as has been demonstrated for some mAbs binding to LFA-1 (40), appears improbable for at least three reasons. (*i*) Anti-CD3 mAb-mediated T-cell activation is not affected by tenascin, whereas some anti-LFA-1 mAbs can inhibit this activation (40). (*ii*) Cell surface expression of LFA-1 is not sufficient for induction of cell clustering by tenascin; LFA-1$^+$ T-cell blasts and the U-937 monocytic cell line (unpublished observations) were not clustered by addition of tenascin, in contrast to EBV-B cells. (*iii*) Immobilized tenascin enhanced the aggregation of nonadherent EBV-B cells. This suggests that the tenascin molecule itself might not be the molecule that clusters the cells.

Delivery of specific signals by ECM proteins or mimicking antibodies upon interaction with specific receptors may result in profound changes in cellular behavior, including proliferation and differentiation (41, 42). We propose that tenascin might also exert its action by delivery of a signal to the cell through a "specific receptor" that affects integrin function through a general mechanism such as cytoskeletal organization or membrane protein motility, thus affecting both cell adhesion properties and T-cell activation.

The immunosuppressive features of tenascin are clearly distinct from those of drugs such as cyclosporin A or steriods or of antibodies against cell surface structures such as LFA-1, CD4, CD8, or MHC molecules. It is now tempting to consider that tenascin expression *in vivo* may provide local fine tuning of the immune response. It may protect tissues undergoing complex changes during inflammation or tissue repair from the unwanted effects of T cells and, conversely, be used by malignant tissues to escape from the immune system (43). The present study also provides further evidence for a regulatory role of ECM proteins in T-cell–accessory cell interactions.

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