Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 6307–6312, June 1997
Immunology

The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells *in situ*

JASON CHESNEY*, MICHAEL BACHER*, ARMIN BENDER†, AND RICHARD BUCALA*‡

*The Picower Institute for Medical Research, Laboratory of Medical Biochemistry, Manhasset, NY 11030; and †The Rockefeller University, Laboratory of Cellular Physiology and Immunology, New York, NY 10021

Communicated by George J. Todaro, University of Washington, Seattle, WA, March 25, 1997 (received for review December 5, 1996)

ABSTRACT **Recent studies have identified a novel population of blood-borne cells, termed fibrocytes, that have a distinct cell surface phenotype (collagen$^+$/CD13$^+$/CD34$^+$/CD45$^+$), rapidly enter sites of tissue injury, and synthesize connective tissue matrix molecules. We found by flow cytometry that purified human fibrocytes express each of the known surface components that are required for antigen presentation, including class II major histocompatability complex molecules (HLA-DP, -DQ, and -DR), the costimulatory molecules CD80 and CD86, and the adhesion molecules CD11a, CD54, and CD58. Human fibrocytes induced antigen-presenting cell-dependent T cell proliferation when cultured with specific antigen and this proliferative activity was significantly higher than that induced by monocytes and nearly as high as that induced by purified dendritic cells. Mouse fibrocytes also were found to express the surface components required for antigen presentation and to function as potent APCs *in vitro*. Mouse fibrocytes pulsed *in vitro* with the HIV-proteins p24 or gp120 and delivered to a site of cutaneous injury were found to migrate to proximal lymph nodes and to specifically prime naive T cells. These data suggest that fibrocytes play an early and important role in the initiation of antigen-specific immunity.**

The skin is a vital barrier to infection or tissue invasion and plays a major role in host immunity (1). Neutrophils and monocytes are an essential first line of defense in the skin and function to kill microorganisms by phagocytosis and by the release of radical species and bactericidal proteins (2). Resident antigen-presenting cells (APCs) such as the Langerhans cell can then initiate antigen-specific responses by processing and presenting microbial antigens to CD4$^+$ T cells by a class II major histocompatibility complex (MHC)-dependent pathway (3).

We recently described a population of novel, blood-borne fibroblast-like cells that rapidly enter sites of tissue injury, synthesize connective tissue matrix, and express fibrogenic cytokines (4). Termed fibrocytes, these cells comprise ≈0.5% of peripheral blood leukocytes and display an adherent, spindle-shaped morphology (ref. 4 and J.C., unpublished observations). Fibrocytes obtained from blood proliferate *in vitro* and synthesize the fibroblast products collagen I, collagen III, and fibronectin, but express several leukocyte-associated cell surface antigens, including the leukocyte common antigen CD45RO, the pan-myeloid antigen, CD13, and the hematopoietic stem cell antigen CD34. Fibrocytes do not express a variety of epithelial, endothelial, or smooth muscle markers and are negative for nonspecific esterases as well as the monocyte/macrophage-specific markers, CD14 and CD16 (4). Fibrocytes also do not express the Langerhans cell marker CD1a, proteins produced by dendritic cells or their precursors (CD25, CD10, and CD38), or the pan-B cell antigen CD19 (3–7).

In the present study, we report that fibrocytes express the cell surface molecules that are required for antigen presentation and are potent inducers of antigen-specific T cell proliferation. Mouse fibrocytes pulsed *in vitro* with foreign antigen and injected into skin were found to migrate to regional lymph nodes and to specifically prime naive T cells. Fibrocytes present in human cutaneous scar tissue also were found by immunohistochemistry to express high levels of the class II MHC molecule HLA-DR *in situ*. These data indicate that fibrocytes may play an early, critical role in the initiation of antigen-specific immunity and significantly expand the importance of these cells in the host response to tissue injury.

MATERIALS AND METHODS

Mice. BALB/c (H-2^d), DBA-2 (H-2^d), C3H/HeJ (H-2^k), and DBA-2 × C3H/HeJ (H-2^{dxk}) mice of both sexes were purchased from The Jackson Laboratory.

Fibrocyte Isolation. Fibrocytes were harvested and cultured as previously described (4). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from human or murine blood by centrifugation over Ficoll/Paque (Pharmacia) following the manufacturer's protocol. After overnight culture on fibronectin-coated plates (human; 6-well plates, 5×10^6 PBMCs per well, murine; 24-well plates, 3×10^6 PBMCs per well) (Becton Dickinson) in DME medium (Life Technologies, Gaithersburg, MD) supplemented with 20% fetal calf serum (FCS; HyClone), the nonadherent cells were removed by a single, gentle aspiration. Following 10 days of continuous culture, the adherent cells were lifted by incubation in cold 0.05% EDTA (Sigma) in PBS (Life Technologies) and were depleted by immunomagnetic selection of contaminating T cells (human: Dynabeads M-450 pan-T, αCD2; murine: pan-T, αCD90, Dynal, Great Neck, NY), monocytes [human: Dynabeads M-450 αCD14; murine: Dynabeads M-450 sheep anti-rat IgG (Dynal), αCD14, rat IgG$_1$, clone rmC5–3 (PharMingen)], and B cells [human: Dynabeads M-450 Pan-B, αCD19; murine: pan-B, αB220 (Dynal)]. Fibrocyte purity was verified to be >95% (70–80% prior to depletion of contaminating cells) by fluorescence-activated cell sorter (FACS) analysis (described below) using both phycoerthrin-conjugated anti-CD34 mAb (Becton Dickinson) and fluorescein-conjugated anti-collagen I mAb (Chemicon) (4). Cell viability was determined to be >90% by trypan blue exclusion.

Dendritic Cell and Monocyte Isolation. Dendritic cells and monocytes were prepared using methods previously described (8, 9). For the isolation of human dendritic cells, T cells first were depleted from PBMCs by rosetting with neuraminadase-treated sheep erythrocytes (Cocalico, Reamstown, PA) (10). The T-depleted mononuclear cells were cultured in RPMI 1640 medium supplemented with 5% human AB serum (6102; Biocell Laboratories) for 2 days and the adherent cells (i.e., monocyte enriched) separated from the nonadherent cells. The adherent monocytes then were lifted from culture wells by incubation in cold PBS/0.5% EDTA and counted. The nonadherent cells were depleted of contaminating monocytes by adherence to human gamma globulin-coated Petri dishes (Calbiochem–Behring) (11). The remaining nonadherent cells then were layered onto 14%

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: APC, antigen-presenting cell; MHC, major histocompatibility complex; FACS, fluorescence-activated cell sorter; PBMCs, peripheral blood mononuclear cells; FCS, fetal calf serum.

‡To whom reprint requests should be addressed. e-mail: rbucala@picower.edu.

metrizamide gradients, centrifuged, and the low-density interface (which contains the dendritic cells) was collected. The dendritic cells were washed twice with PBS, enumerated (purity > 70%), and cultured for further studies.

FACS Analysis. A total of 2×10^5 cells per sample were washed twice in PBS containing 0.1% sodium azide (Sigma) and 1% BSA (Sigma) (FACS medium). The cells were resuspended in 25 μl of diluted antibody (in PBS) and incubated for 30 min on ice (12). The cells then were washed twice in PBS and resuspended in 200 μl of FACS medium. At least 10,000 cells were analyzed on a FACScan instrument (Becton Dickinson). Human cells were stained with the following phycoerythrin or fluorescein-conjugated mAbs: anti-CD11a (clone G-25.2), anti-CD54 (clone LB-2), anti-CD58 (clone L306.4), anti-CD80 (clone L307.4) (each from Becton Dickinson); and anti-HLA-DP (clone HI43), anti-HLA-DQ (clone TU169), anti-HLA-DR (clone TU36), and anti-CD86 (clone IT2.2) (each from PharMingen). Murine fibrocytes were stained with the following phycoerythrin or fluorescein-conjugated mAbs: anti-I-A^d (clone AMS-32.1), anti-CD11a (clone M17/4), anti-CD54 (clone 3E2), anti-CD86 (clone GL1) (each from PharMingen), and anti-I-E^d (clone E-E-D6) (Accurate Laboratories, Westbury, NY). Direct-conjugated isotype controls and cell only samples were analyzed with each antibody.

Human Antigen-Dependent Autologous T Cell Proliferation Assay. Three human blood donors were boosted intramuscularly with 4 units of tetanus toxoid (Connaught Laboratories). One month later, the PBMCs were isolated from the peripheral blood. T cells were isolated by high-affinity negative selection (human T Cell Enrichment Column, R & D Systems). FACS analysis of anti-CD3 (PharMingen) labeled cells indicated that 85–95% of all cells recovered were $CD3^+$. APCs were prepared as described above and treated with 25 μg/ml mitomycin C (Sigma) in RPMI medium 1640 containing 10% human AB serum (RPMI/10% HS) for 30 min and then washed five times with RPMI/10% HS. For each assay, the T cells (2×10^5) were incubated with mitomycin C-treated autologous fibrocytes, monocytes, or dendritic cells at various T cell:APC ratios in the presence of 2 μg/ml tetanus toxoid in RPMI/10% HS (13). After 4, 5, and 6 days of coculture, the proliferative activity was measured over 12 hr by the incorporation of [^{3}H]thymidine (4 μCi/ml; 1 Ci = 37 GBq) into DNA as measured by liquid scintillation counting. Controls that were included in each experiment were APCs alone, T cells alone, APCs + tetanus toxoid, T cells + tetanus toxoid, and APC + T cells. Mixed leukocyte reactions were conducted similarly except that the fibrocytes and T cells were isolated from allogeneic donors and no antigen was included in the coculture (13). Statistical significance was assessed by two sample *t* tests (assuming unequal variances) (14).

Antibody Neutralization Studies. The effect of the following neutralizing mAbs on fibrocyte antigen presentation was determined: anti-CD11a, anti-CD54, anti-CD86, and anti-HLA-DR. The helper assay was conducted as described above except that 5 μg/ml of receptor-specific or isotype control mAb was added to a 20:1 T cell:fibroctye coincubation just prior to the addition of tetanus toxoid. Control studies established that these accessory cell receptor-specific mAbs have no effect on human T cell proliferation induced by phorbol 12-myristate 13-acetate (1 ng/ml) plus ionomycin (5 ng/ml) (Sigma) (data not shown).

Murine Antigen-Dependent Autologous T Cell Proliferation Assay. BALB/c mice were immunized by i.p. injection of 100 μg of native p24 or gp120 (purified from $HIV\text{-}1_{IIIB}$ infected H9 cells, Advanced Biotechnologies, Columbia, MD) emulsified at 1 mg/ml with Freund's complete adjuvant. After 14 days, the T cells were purified from spleens by chromatography over a T cell enrichment column (R & D Systems). Mouse fibrocytes were purified from peripheral blood as described above and treated with 25 μg/ml mitomycin C (Sigma) in RPMI medium containing 10% FCS (RPMI/10% FCS) for 30 min and then washed 5 times with RPMI/10% FCS. For each assay, the T cells (2×10^5) were incubated with mitomycin C-treated fibrocytes at various ratios in the presence of 2 μg/ml p24 or gp120 in RPMI/10% FCS (15). After 5 days of coculture, the proliferative activity was measured over 12 hr by the incorporation of [^{3}H]thymidine (4 μCi/ml) into DNA as measured by liquid scintillation counting. Controls that were included in each experiment were fibrocytes alone, T cells alone, fibrocytes + antigen, T cells + antigen, and fibrocytes + T cells.

Priming of Naive T Cells *in Situ* with Antigen-Pulsed Murine Fibrocytes. Following the methods described by Steinman *et al.* (16), purified BALB/c fibrocytes were cultured for 3 days with 50 μg/ml of p24 or gp120 in DME/20% FCS, washed five times in PBS, and injected intradermally (5×10^4 cells in 20 μl PBS) into the right rear footpad. The proximal popliteal lymph nodes were explanted 5 days later and cell suspensions prepared by teasing with fine forceps. A total of 2×10^5 lymph node cells per well were cultured with 50 μg/ml of antigen in Click's medium (GIBCO) supplemented with 1% heat-inactivated mouse serum (Sigma) and 50 μM 2-mercaptoethanol (Sigma) for 72 hr. The proliferative activity was measured over the last 12 hr of culture by the incorporation of [^{3}H]thymidine (4 μCi/ml) into DNA. Proliferating cells were identified to be primarily $CD4^+$ T cells through depletion of $CD4^+$ T cells by immunomagnetic selection (Dynabeads M-450 L3T4, CD4, Dynal) just prior to liquid scintillation counting. In certain experiments, DBA-2 × C3H/HeJ F_1 mice ($H\text{-}2^{dxk}$) were injected with pulsed fibrocytes from either parent strain (DBA-2, $H\text{-}2^d$ or C3H/HeJ, $H\text{-}2^k$), and, 5 days later, the proximal popliteal lymph nodes were isolated and depleted of endogenous class II MHC^+ APCs by immunomagnetic selection (Dynabeads M-450 sheep anti-rat IgG, Dynal, anti-murine class II MHC, rat IgG_{2b}, clone ER-TR 3, Accurate Laboratories). APC-depleted lymph node cells (1×10^5) then were cocultured with 1×10^5 mitomycin C-treated F_1 or parent spleen cells as APC with or without gp120 for 72 hr. The proliferative activity was measured as described above.

Cell Migration Studies. Mouse fibrocytes purified from peripheral blood were stained with the PKH26-GL red fluorescent cell linker compound (Sigma) following the manufacturer's protocol. Prior to staining, a subset of fibrocytes was fixed in 0.1% glutaraldehyde for 30 min at room temperature and washed three times in PBS, 1% FCS. After staining, the cells were washed five times in PBS, counted, and assessed for labeling efficiency by fluorescence microscopy (≈60% for nonfixed, ≈40% for fixed) and viability by trypan blue exclusion (≈80% for nonfixed, 0% for fixed). Labeled cells (3×10^4) in 20 μl PBS were administered intradermally into the right rear footpad of BALB/c mice. After 24 hr, the mice were killed by CO_2 asphyxiation and the popliteal lymph nodes were removed and the cells dissociated. The entire resultant cell suspension from each lymph node (3×10^6 cells in 100 μl PBS) then was examined at ×400 by fluorescence microscopy and the labeled cells enumerated (all fields). Data are expressed as mean ± SD ($n = 3$).

Immunohistochemistry. Human cutaneous scar tissue specimens were obtained 2–3 weeks after initial injury from individuals without systemic disease. The tissue was fixed in 3.5% paraformaldehyde, sectioned, and processed for immunohistochemical analyses. After blocking endogenous peroxidases with H_2O_2 (3%), the deparaffinized sections were incubated with an anti-CD34 mAb (1:50 dilution) (clone QB-END/10) (Accurate, Westbury, NY) or an IgG_1 isotype control. After washing, an immunoperoxidase-linked secondary antibody (Dako) was added, followed by diaminobenzidene as substrate. The sections then were labeled with anti-HLA-DR mAb (Pharmacia) or an IgG_{2b} isotype control, incubated with an alkaline phosphatase-linked secondary antibody, and developed with new fuchsin (Dako) as substrate. Control sections stained with an isotype control or without primary antibody showed no immunoreactivity.

RESULTS

Antigen-presenting cells are known to express cell surface proteins that enhance T cell stimulation in response to the MHC-peptide complex (reviewed in ref. 17). Specific receptor:coreceptor pairs which have been found to be essential include CD11a/18-CD54, CD58-CD2, and CD86-CD28. To characterize the ability of human fibrocytes to activate T cells, we first examined the expression of these molecules on the surface of purified, human fibrocytes. As shown in Fig. 1, fibrocytes isolated from peripheral blood express high levels of the adhesion molecules, CD11a, CD54, and CD58. The level of expression is similar to that observed in purified, peripheral blood monocytes that had been cultured for 36 hr. Fibrocyte expression of the class II MHC molecules HLA-DP and HLA-DQ is higher in fibrocytes than in monocytes, although the expression of HLA-DR appears equivalent in both cell types. Fibrocytes express the costimulatory molecule CD86 (B7–2) at a level similar to that of monocytes. Fibrocytes also weakly express the costimulatory molecule CD80 (B7–1) (data not shown). These data indicate that fibrocytes constitutively produce the full complement of surface proteins that have been shown in other cell types to be necessary for antigen presentation.

To assess the potential capacity of fibrocytes to present antigen, we next examined the ability of purified human fibrocytes to activate allogeneic T cells in a mixed leukocyte reaction. Various concentrations of mitomycin C-treated fibrocytes were cocultured with allogeneic T cells for 96 hr and the T cell proliferative activity assessed by the incorporation of [^{3}H]thymidine into DNA. Fibrocytes thus were found to induce a significant T cell activation response (5×10^3 fibrocytes + 2×10^5 T cells = 70, 321 ± 10, 111 cpm, versus <300 cpm for T cells or fibrocytes alone, $n = 3$ experiments, $P < 0.001$). We then proceeded to examine the capacity of fibrocytes to present soluble antigen in an autologous, T cell proliferation assay. T cells were purified from the peripheral blood of tetanus toxoid-immunized individuals and stimulated with tetanus toxoid *in vitro* together with fibrocytes as APCs. As shown in Fig. 2*A*, mitomycin C-treated fibrocytes induced a powerful, antigen-dependent T cell proliferative response when coincubated with autologous T cells. T cell proliferation, as measured by [^{3}H]thymidine incorporation, was greatest when 3,750 fibrocytes were coincubated with 2×10^5 T cells (30,475 ± 3,692 cpm). The level of T cell activation was not significantly different on days 4, 5, or 6 after the start of the coincubation. Fixation of fibrocytes with paraformaldehyde prior to coincubation with T cells and tetanus toxoid did not induce antigen-dependent proliferation (1,050 ± 378 cpm, 3,750 fibrocytes + 2×10^5 T cells), suggesting that the intracellular processing of tetanus toxoid is a required step in fibrocyte antigen presentation.

For comparison purposes, we also examined the antigen-presenting capacity *in vitro* of two other purified cell types: monocytes and dendritic cells (Figs. 2 *B* and *C*). Using the same preparation of purified, autologous T cells, the peak antigen-dependent T cell proliferation response induced by monocytes was significantly lower than that induced by fibrocytes (14,555 ± 5,404 cpm, $P < 0.005$). Furthermore, more monocytes than fibrocytes (10,000 monocytes versus 3,750 fibrocytes) were required to reach maximum stimulation in the coincubation assay.

The peak antigen-dependent T cell proliferation induced by dendritic cells was higher than that induced by fibrocytes (37,348 ± 2,019 cpm, $P < 0.01$). However, more dendritic cells than fibrocytes (10,000 dendritic cells vs. 3,750 fibrocytes) were required to achieve this level of proliferative activity. Interestingly, an inhibition of T cell stimulatory activity with higher APC numbers was observed with monocytes and fibrocytes, but not with dendritic cells.

We next examined the functional requirement of HLA-DR, CD86, and the coligands, CD54 and CD11a, for antigen presentation by human fibrocytes. Neutralizing mAbs were added to the fibrocyte, T cell cocultures prior to the addition of tetanus toxoid and their effect on antigen-dependent T cell proliferation measured. As shown in Fig. 3, fibrocyte-induced T cell proliferation was inhibited significantly by neutralizing mAbs to HLA-DR, CD86, CD11a, or CD54. Taken together, these data indicate that

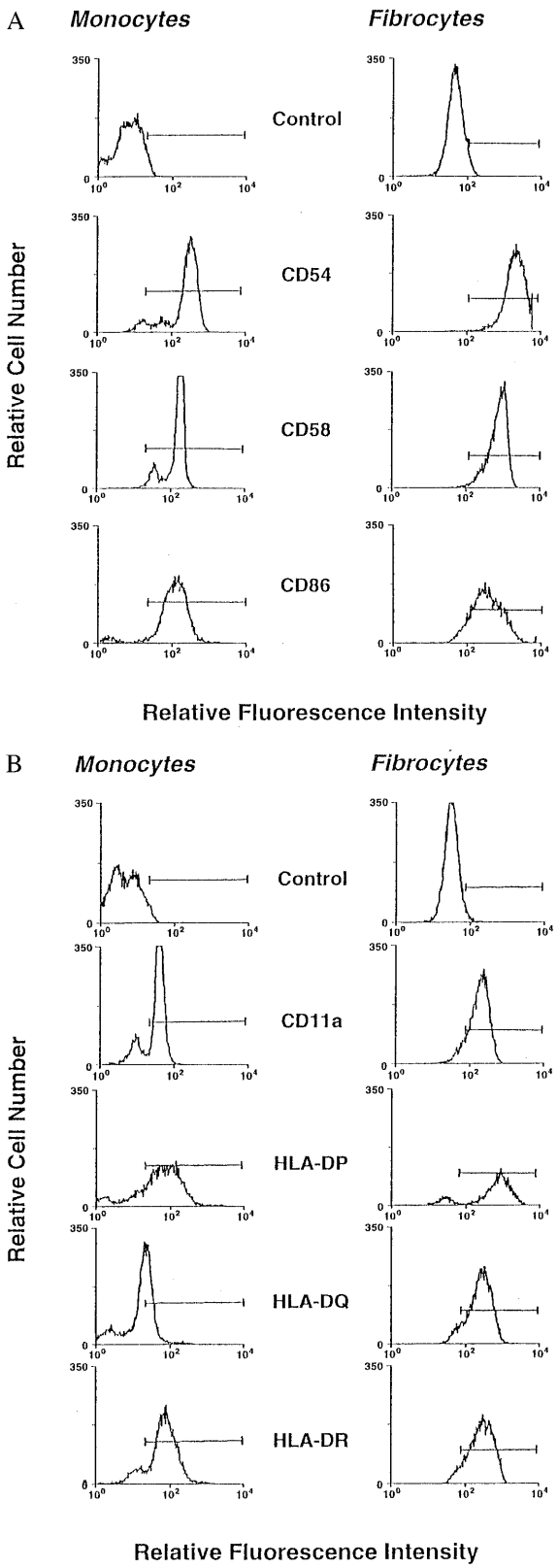

FIG. 1. Accessory molecule expression by human fibrocytes and monocytes. Purified, human fibrocytes or monocytes were incubated with phycoerythrin-conjugated (*A*) or fluorescein isothiocyanate-conjugated (*B*) mAbs and analyzed by flow cytometry as described. The horizontal line in each panel marks fluorescence intensity greater than the background staining that was observed with an isotype control mAb.

fibrocyte antigen presentation is class II MHC dependent and requires both costimulation through CD86 and adhesion through a CD11a:CD54 interaction.

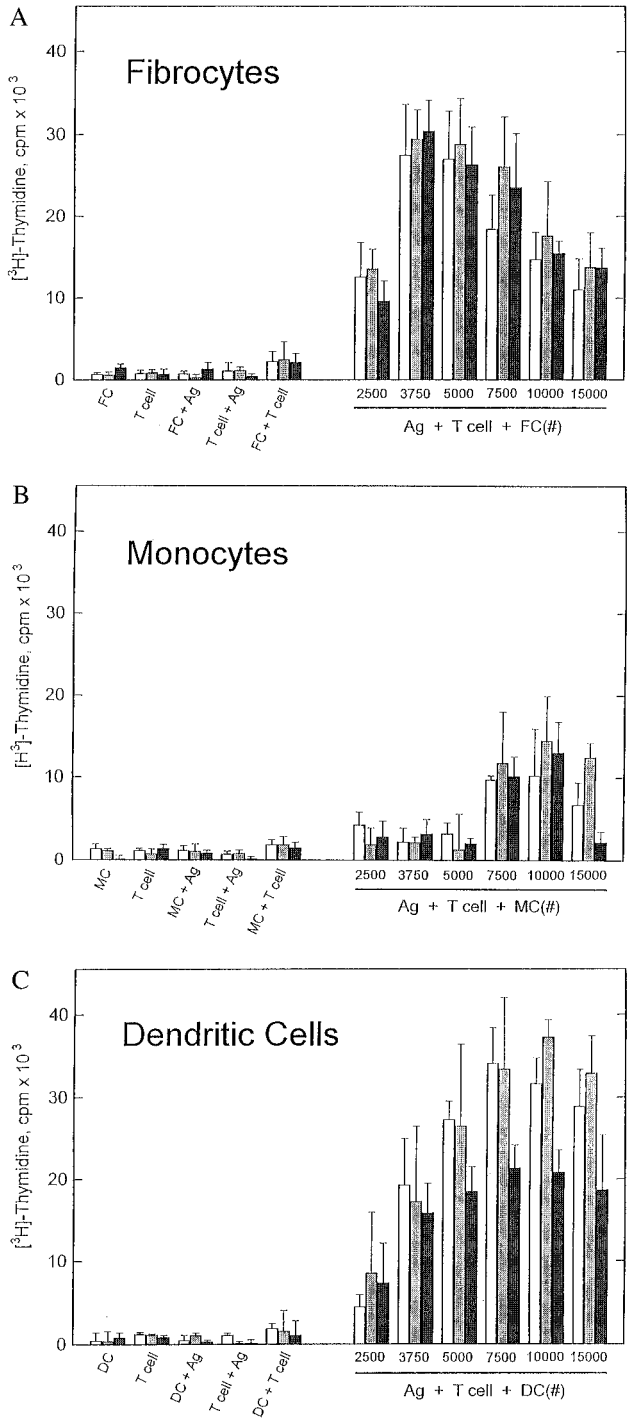

FIG. 2. Functional comparison of human fibrocyte, monocyte, and dendritic cell antigen presentation *in vitro*. Human T cells (2×10^5) purified from a tetanus toxoid-immunized individual were incubated with 2 μg/ml tetanus toxoid together with various numbers of autologous fibrocytes (*A*), autologous monocytes (*B*), or autologous dendritic cells (*C*). After incubation for 4 (□), 5 (▨), and 6 (■) days, the cultures were pulsed for 12 hr with 4 μCi/ml [^{3}H]thymidine and cell proliferation analyzed by liquid scintillation counting. Controls are illustrated on the left side of each figure: APCs alone (FC, fibrocytes; MC, monocytes; DC, dendritic cells), T cells alone, APCs + Ag (tetanus toxoid), T cells + Ag (tetanus toxoid), and APCs + T cells. Data are expressed as mean ± SD and are representative of one experiment that was performed three times.

To examine more closely the ability of fibrocytes to present antigen *in vivo*, we also studied the functional responses of fibrocytes purified from mouse blood. Mouse fibrocytes share many of the same phenotypic properties with human fibrocytes and express both type I collagen and CD34 (4). By flow cytometry analysis, mouse fibrocytes also were found to express the class II MHC molecules, I-A and I-E, the adhesion molecules, CD11a and CD54, and the costimulatory molecule, CD86 (data not shown).

Mouse fibrocytes also induced a significant antigen-dependent T cell proliferation response when coincubated with primed, autologous T cells. T cells were purified from the peripheral blood of BALB/c mice immunized with the HIV proteins p24 and gp120 and stimulated *in vitro* with p24 or gp120 utilizing fibrocytes as APCs (Fig. 4). T cell proliferation, as measured by [^{3}H]thymidine incorporation, was greatest when 15,000 murine fibrocytes were coincubated with 2×10^5 T cells (p24: 22,272 ± 7,751 cpm, and gp120: 24,276 ± 2,680).

Although several cell types have been shown to be capable of presenting antigen to memory T cells, the priming of naive T cells has been considered to be a specialized function of "professional" APCs, particularly dendritic cells (16, 25). To test the ability of mouse fibrocytes to prime naive lymph node T cells *in vivo*, we pulsed mouse fibrocytes with p24 or gp120 *in vitro* and injected them intra-dermally into the rear foot pad of unprimed BALB/c mice. Five days later, the popliteal lymph nodes were removed and the constituent cells tested for a restimulation proliferative response. Injected, antigen-pulsed fibrocytes thus were found to induce a strong T cell proliferative response (Fig. 5). This response was specific for the priming antigen (p24 or gp120) and consisted predominantly of CD4$^+$ T cells since immunodepletion of this T cell subset resulted in no detectable proliferation signal.

That antigen-pulsed fibrocytes were not simply transferring antigen to other host APC types was established by experiments in which antigen-pulsed fibrocytes from two parent mouse strains were injected into F_1 offspring mice. The T cell reactivity of F_1 offspring is confined predominantly to antigens presented by one of the parental strains, and the priming and restimulation APCs must necessarily share the same haplotype (16, 18, 19). DBA-2 × C3H/HeJ F_1 mice (H-2^{dxk}) thus were injected with pulsed fibrocytes from either parent (DBA-2, H-2^d or C3H/HeJ, H-2^k) and, five days later, the proximal popliteal lymph nodes were isolated and depleted of endogenous class II MHC$^+$ APCs by immunomagnetic selection. The F_1 APC-depleted lymph node cells then were cultured with F_1 (H-2^{dxk}) or parent strain (H-2^d or H-2^k) spleen cells as the source of APC, with or without gp120. As shown in Fig. 6, the F_1 APC-depleted lymph node cells were reactive to

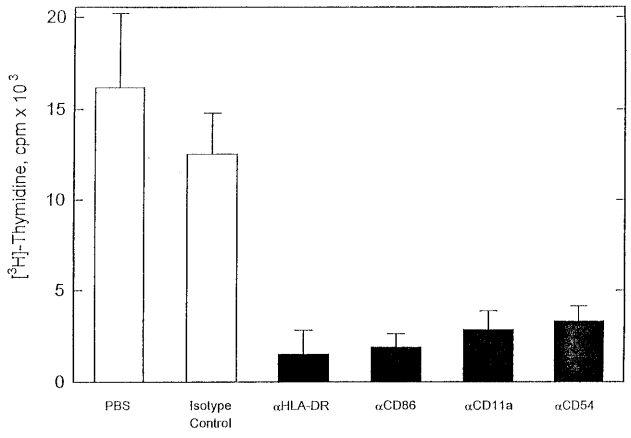

FIG. 3. Effect of neutralizing anti-HLA-DR, anti-CD86, anti-CD54, or anti-CD11a mAbs on fibrocyte antigen presentation *in vitro*. Receptor-specific or isotype control (5 μg/ml) mAb were added to a 20:1 autologous T cell:fibrocyte coincubation prior to the addition of tetanus toxoid. The antigen-dependent T cell proliferation assay was conducted as in Fig. 2. Data are expressed as mean ± SD and are representative of one experiment that was performed three times.

antigen in the presence of the F_1 restimulation APCs when priming was performed with fibrocytes from either parental strain. However, if a parental strain was used as the source of restimulation APCs, the F_1 APC-depleted lymph node cells would only proliferate if the priming fibrocytes were from the same parental strain. These data indicate that fibrocyte priming and APC restimulation of sensitized T cells occurs only in the setting of a shared MHC haplotype. Thus, fibrocytes do not function merely to deliver antigen to other APCs, but rather act to directly sensitize naive T cells in a MHC-specific manner.

We also sought to quantify the migration of fibrocytes from a site of peripheral injury into regional lymph nodes. Fluorescent-labeled murine fibrocytes were administered intradermally into the rear footpads of mice and, 24 hr later, the popliteal lymph nodes were removed, dissociated, and examined by fluorescence microscopy. Approximately 5% of the labeled fibrocytes that were injected were observed in the popliteal lymph node (1,567 ± 251 of 30,000 fibrocytes injected, $n = 3$ mice). By contrast, fibrocytes which were glutaraldehyde-fixed prior to the injection were not observed to migrate into the lymph node [<10 cells (detection limit) of 30,000 fibrocytes injected, $n = 3$ mice].

Additional evidence for a role of fibrocyte antigen presentation *in vivo* was obtained by immunohistochemical studies of human cutaneous scar specimens. Human cutaneous scar specimens were examined for the presence of spindle-shaped cells that coexpressed CD34 and HLA-DR. Fig. 7 shows a subdermal region in which numerous connective tissue matrix-associated inflammatory cells were found to stain positively for both CD34 and HLA-DR. Under high power, the expression of both molecules is evident and appears to localize predominately to cytoplasmic extensions. The expression of HLA-DR by human fibrocytes *in vivo* suggests that these cells may be active participants in the antigen presentation processes associated with wound repair.

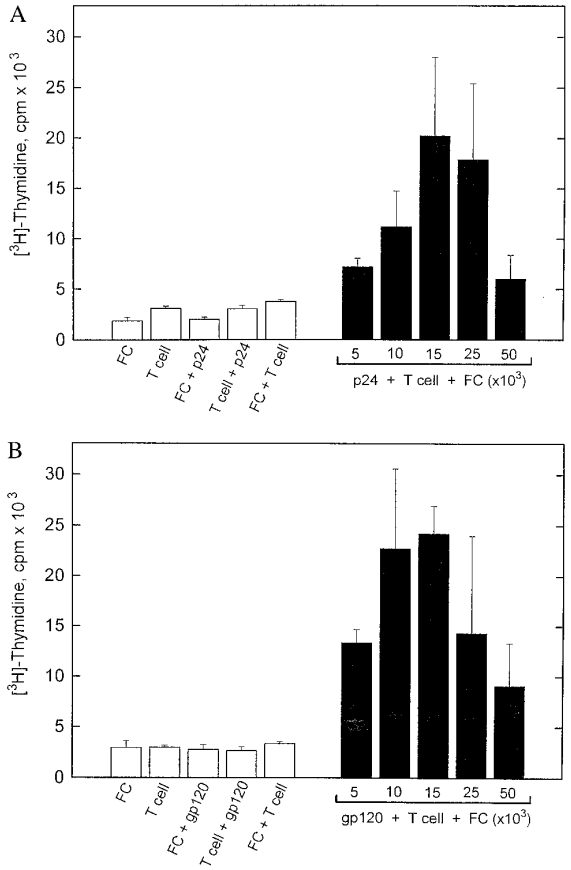

FIG. 4. Murine fibrocyte antigen presentation *in vitro*. Murine T cells (2×10^5) purified from the spleens of p24 (*A*) or gp120 (*B*) immunized BALB/c were incubated with 2 μg/ml p24 or gp120 together with various numbers of mitomycin C-treated autologous fibrocytes. After incubation for 5 days, the cultures were pulsed for 12 hr with 4 μCi/ml [^{3}H]thymidine and cell proliferation analyzed by liquid scintillation counting. Controls are illustrated on the left side of each figure. Data are expressed as mean ± SD and are representative of one experiment that was performed three times.

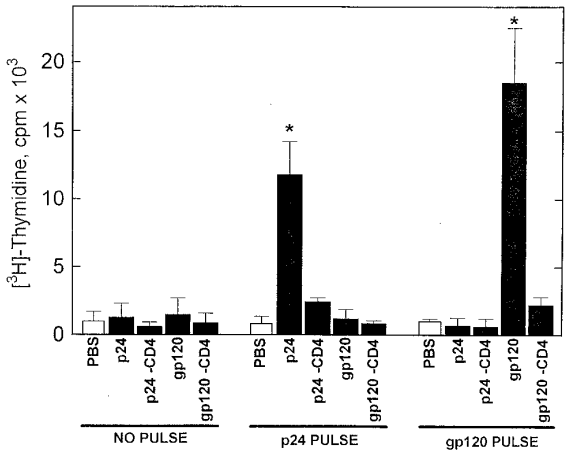

FIG. 5. Priming of naive T cells *in situ* with antigen-pulsed murine fibrocytes. Purified BALB/c mouse fibrocytes were cultured for 3 days without (NO PULSE), or with 50 μg/ml of p24 (p24 PULSE), or gp120 (gp120 PULSE) in DME/20% FCS, washed, and injected i.d. (5×10^4 cells) into the right rear footpad. The proximal popliteal lymph nodes were explanted 5 days later, dissociated, and cultured with PBS or 50 μg/ml of p24 or gp120 for 72 hr. To verify the proliferating cell type, $CD4^+$ T cells were depleted by immunomagnetic selection just prior to liquid scintillation counting (p24-CD4, gp120-CD4). The proliferative activity was measured over the last 12 hr of culture by [^{3}H]thymidine incorporation. Data are expressed as mean ± SD and are representative of one experiment that was performed three times.

DISCUSSION

Fibrocytes are unique among connective tissue cells in that they express collagens but circulate in blood and display the hematopoietic/leukocyte cell surface marker CD34. Studies employing implantable wound chambers have established that fibrocytes may account for as many as 10% of the cells that infiltrate sites of acute tissue injury (4). Immunohistochemical

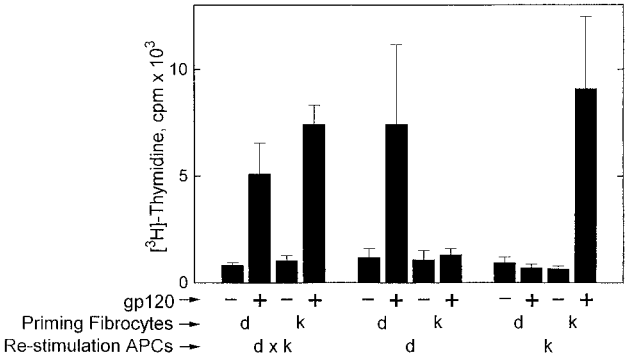

FIG. 6. Requirement of shared MHC haplotype between priming fibrocyte and restimulation APC. DBA-2 × C3H/HeJ F_1 mice (H-2^{dxk}) were injected with antigen-pulsed fibrocytes from either parent strain DBA-2, H-2^d (d) or C3H/HeJ, H-2^k (k). Five days later, the proximal popliteal lymph node cells were isolated and depleted of endogenous class II MHC^+ APCs by immunomagnetic selection. APC-depleted lymph node cells (1×10^5) then were cocultured with 1×10^5 mitomycin C-treated F_1 (dxk) or parental spleen cells (d or k) as restimulation APCs, with or without gp120. The proliferative activity was measured over the last 12 hr of culture by [^{3}H]thymidine incorporation. Data are expressed as mean ± SD and are representative of one experiment that was performed three times.

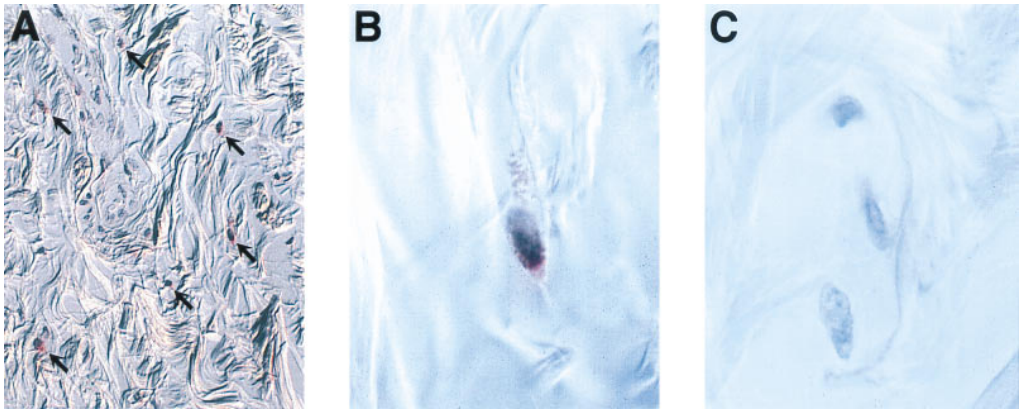

FIG. 7. Coexpression of CD34 and HLA-DR by cells in a human cutaneous tissue scar. Skin specimens were fixed, sectioned, and labeled with both anti-CD34 mAb and anti-HLA-DR mAb. Binding was detected with an immunoperoxidase-linked (anti-CD34) and an alkaline phosphatase-linked (anti-HLA-DR) secondary antibody. The anti-CD34 positive areas appear brown and granular and the anti-HLA-DR positive regions stain red. The isotype control displays no immunoreactivity. [×200 (*A*); ×1,000 (*B* and *C*).]

analyses of tissues undergoing fibrosis and tissue remodeling also have identified fibrocytes to be present within areas of extracellular matrix deposition, providing direct evidence for the participation of fibrocytes in the host repair response to tissue injury. Utilizing sex-mismatched, bone marrow chimeric mice together with DNA amplification of the male-specific SRY gene, it was found that circulating fibrocytes do not appear to originate from radiosensitive, hematopoietic stem cells but arise instead from a radioresistant, bone marrow or other tissue source. Importantly, fibrocytes do constitutively express the leukocyte common antigen, CD45, a marker of bone marrow-derived cells (4).

In the present study, we show that peripheral blood fibrocytes: (*i*) express the full complement of surface proteins required for antigen presentation, (*ii*) are potent stimulators of antigen-specific T cells *in vitro*, and (*iii*) migrate to lymph nodes and sensitize naive T cells *in situ*. The constitutive expression by fibrocytes of the surface proteins known to be necessary for antigen presentation contrasts with what has been described for tissue fibroblasts that require activation by interferon-γ to express measurable quantities of HLA-DR (20). Although several tissue-derived cells have been shown to be capable of presenting antigen to memory T cells, including dermal fibroblasts, endothelial cells, and melanocytes (20, 23, 24, respectively), the sensitization of naive T cells has been considered to be a particular function of dendritic cells (16, 25). Fibrocytes are distinct from dendritic cells and their precursors not only in their growth properties (fibrocytes are an adherant, proliferating cell population whereas dendritic cells are nonadhering and poorly proliferating) but also in their surface protein expression (collagen$^+$/CD13$^+$/CD34$^+$/CD25$^-$/CD10$^-$/CD38$^-$). That fibrocytes also have a specialized and potent antigen presentation activity suggests that they may play a critical role in the initiation of immunity during tissue injury and repair.

HLA-DR expression is considered a prerequisite for antigen presentation *in vivo* (20) and human fibrocytes participating in cutaneous tissue repair were found to express high levels of HLA-DR *in situ*. Although these data do not establish conclusively that fibrocytes actively present antigen in human tissue, they are consistent with a role for fibrocytes in the immune response associated with skin injury. In mice, an appreciable portion (5%) of fibrocytes were found to home to regional lymph nodes after intradermal injection into skin. Fibrocytes may function *in vivo* to capture foreign proteins at sites of tissue injury and to migrate into regional lymph nodes for the purpose of sensitizing naive T cells and/or activating memory T cells. Fibrocytes also have been shown to secrete a number of inflammatory cytokines *in vitro* and are a particularly abundant source of the potent CD4$^+$ T cell chemoattractants, macrophage inflammatory proteins (MIP-1α and 1β) (ref. 21, unpublished work). The entry of CD4$^+$ T cells into areas of tissue damage is considered to be an essential requirement for the generation of an antigen-specific immune response (22). Fibrocytes thus may act to not only activate but to also recruit CD4+ T cells into the tissue repair microenvironment.

The ability of fibrocytes to secrete collagen and other matrix components suggests that these cells may play an important role in certain autoimmune and connective tissue disorders. A persistent fibrocyte:T cell activation response may lead to pathologically significant fibrosis and scarring, such as occurs in scleroderma, schistosomiasis, or graft vs. host disease. Schistosomiasis, for instance, is characterized by a fibrosing, granulomatous reaction directed against schistosome eggs that can lead ultimately to fulminant liver failure (26). Fibrocytes have been identified recently to colocalize to areas of matrix deposition in *S. mansoni* infected livers (J. Chesney, *in preparation*). Further investigation into the normal and pathological role of this novel cell population may provide important insight into the control of the fibrotic responses associated with inflammation. Additionally, fibrocytes may find clinical utility as a readily accessible source of APCs that are capable of initiating and promoting T cell immunity.

We thank Nina Bhardwaj for helpful discussions and Cathy Rapelje for expert assistance with the FACS analyses. A.B. is supported by Deutsche Forschungsgemeinschaft BS 1609/1-1.

1. Mast, B. A. (1992) in *Wound Healing: Biochemical and Clinical Aspects*, eds. Cohen, I. K., Diegelmann, R. F. & Lindblad, W. J. (Saunders, Philadelphia), pp. 344–355.
2. Davidson, J. M. (1992) in *Inflammation: Basic Principles and Clinical Correlates*, eds. Gallin, J. I., Goldstein, I. M. & Snyderman, R. (Raven, New York), pp. 809–819.
3. Chu, T. & Jaffe, M. B. (1994) *Br. J. Cancer Suppl.* **23,** S4–S10.
4. Bucala, R., Spiegel, L. A., Chesney, J. A., Hogan, M. & Cerami, A. (1994) *Mol. Med.* **1,** 71–81.
5. Freudenthal, P. S. & Steinman, R. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7698–7702.
6. Galy, A., Travis, M., Cen, D. & Chen, B. (1995) *Immunity* **3,** 459–473.
7. Barclay, A. N., Beyers, A. B., Birkeland, M. L., Brown, M. H., Davis, S. J., Somoza, C. & Williams, A. F. (1993) *The Leukocyte Antigen Facts Book* (Academic, New York), pp. 142–143.
8. Bhardwaj, N., Bender, A., Bonzalez, N., Bui, L. K., Garrett, M. C. & Steinman, R. M. (1994) *J. Clin. Invest.* **94,** 797–807.
9. Bender, A., Bui, L. K., Feldman, M. A., Larsson, M. & Bhardwaj, N. (1995) *J. Exp. Med.* **182,** 1663–1671.
10. Bhardwaj, N., Young, J. W., Nisanian, A. J., Baggers, J. & Steinman, R. M. (1993) *J. Exp. Med.* **178,** 633–642.
11. Young, J. W. & Steinman, R. M. (1988) *Cell. Immunol.* **111,** 167–182.
12. Loken, M. R. & Wells, D. A. (1994) in *Flow Cytometry*, ed. Ormerod, M. G. (Oxford Univ. Press, Oxford), pp. 67–92.
13. Jasmes, S. P. (1991) in *Current Protocols in Immunology*, eds. Coligan, J. E., Kruisbeek, A. M., Marguiles, D. H., Shevach, E. M. & Strober, W. (Wiley, New York), pp. 7.10.1–7.11.4.
14. Zar, J. H. (1984) *Biostatistical Analysis* (Prentice–Hall, Englewood Cliffs, NJ).
15. Kruisbeek, A. D. & Shevach E. (1991) in *Current Protocols in Immunology*, eds. Coligan, J. E., Kruisbeek, A. M., Marguiles, D. H., Shevach, E. M. & Strober, W. (Wiley, New York), pp. 3.12.1–3.12.14.
16. Inaba, K., Metlay, J. P., Crowley, M. T. & Steinman, R. M. (1990) *J. Exp. Med.* **172,** 631–640.
17. Clark, E. A. & Ledbetter, J. A. (1994) *Nature (London)* **367,** 425–428.
18. Sprent, J. (1978) *J. Exp. Med.* **147,** 1159.
19. Sprent, J. (1978) *J. Exp. Med.* **147,** 1142.
20. Geppert, T. D. & Lipsky, P. E. (1985) *J. Immunol.* **135,** 3750–3762.
21. Schall, T. J., Bacon, K., Camp, R. D. R., Kaspari, J. W. & Goeddel, D. V. (1993) *J. Exp. Med.* **177,** 1821–1825.
22. Wahl, L. M. and Wahl, S. M. (1992) in *Wound Healing: Biochemical and Clinical Aspects*, eds. Cohen, I. K., Diegelmann, R. F. & Lindblad W. J. (Saunders, Philadelphia), pp. 40–62.
23. Pober, J. S., Gimbrone, M. A., Cotran, R. S., Reiss, C. S., Burakoff, S. J., Fiers, W. & Kenneth, A. (1983) *J. Exp. Med.* **157,** 1339–1353.
24. Poole, I. C., Mutis, T., Rene, M., Wijngaard, G. J., Westerohof, W., Ottenhoff, T., Vries, R. & Das P. K. (1993) *J. Immunol.* **12,** 7284–7292.
25. Levin, D., Constant, S., Pasqualini, T., Flavell, R. & Bottomly, K. (1993) *J. Immunol.* **12,** 6742–6750.
26. Warren, K. S., Domingo, E. O. & Cowan, R. B. T. (1967) *Am. J. Pathol.* **51,** 735–756.