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Adult Human Fibroblasts Are Potent Immunoregulatory Cells and Functionally Equivalent to Mesenchymal Stem Cells

Muzlifah A. Haniffa,†† Xiao-Nong Wang,* Udo Holtick,* Michelle Rae,* John D. Isaacs, † Anne M. Dickinson,* Catharien M. U. Hilkens,2† and Matthew P. Collin2,3* 

Bone marrow mesenchymal stem cells (MSC) have potent immunosuppressive properties and have been advocated for therapeutic use in humans. The nature of their suppressive capacity is poorly understood but is said to be a primitive stem cell function. Demonstration that adult stromal cells such as fibroblasts (Fb) can modulate T cells would have important implications for immunoregulation and cellular therapy. In this report, we show that dermal Fb inhibit allogeneic T cell activation by autologously derived cutaneous APCs and other stimulators. Fb mediate suppression through soluble factors, but this is critically dependent on IFN-γ from activated T cells. IFN-γ induces IDO in Fb, and accelerated tryptophan metabolism is at least partly responsible for suppression of T cell proliferation. T cell suppression is reversible, and transient exposure to Fb during activation reprograms T cells, increasing IL-4 and IL-10 secretion upon restimulation. Increased Th2 polarization by stromal cells is associated with amelioration of pathological changes in a human model of graft-vs-host disease. Dermal Fb are highly clonogenic in vitro, suggesting that Fb-mediated immunosuppression is not due to outgrowth of rare MSC, although dermal Fb remain difficult to distinguish from MSC by phenotype or transdifferentiation capacity. These results suggest that immunosuppression is a general property of stromal cells and that dermal Fb may provide an alternative and accessible source of cellular therapy. The Journal of Immunology, 2007, 179: 1595–1604.

stromal cells in the bone marrow (BM) were first described in 1968 (1) as the primitive mesenchymal companions of hemopoietic stem cells. Mesenchymal stem cells (MSC) from the BM were subsequently shown to be multipotential by their ability to differentiate into bone, cartilage, and fat (2). More recently, MSC were demonstrated to have pleiotropic immunomodulatory effects in vitro including direct suppression of allogeneic and mitogenic T cell proliferation (3–6), induction of T cell anergy (7) or apoptosis (8), modulation of cytokine production (9), and inhibition of dendritic cell (DC) maturation (10, 11). Immunomodulation is not Ag specific and is promoted by close contact but reported to be mediated by a number of soluble factors including TGF-β, tryptophan metabolites, and PGE2 (4, 6, 7, 9, 12). Several animal models lend further credence to the therapeutic potential of MSC (13–16), although this is judiciously tempered by reports of increased graft rejection (17), sarcoma formation (18), and low efficacy in one graft-vs-host disease (GVHD) model (19).

Recent case reports and early-phase clinical trials have suggested that i.v. cellular therapy with MSC isolated from BM by tissue culture plastic adherence followed by in vitro expansion can induce striking remissions in patients with severe GVHD after hemopoietic stem cell transplantation (Refs. 20–23; reviewed in Refs. 24 and 25).

It is widely believed that generic stromal cells such as fibroblasts (Fb) do not share the immunosuppressive effects of MSC (26). In practice, it is difficult to distinguish MSC from Fb by surface phenotype; indeed, the Ags used to define MSC for clinical trial are all found on Fb (25, 27). Transdifferentiation is said to be a key characteristic of MSC (1, 2, 28, 29) and the immunosuppressive properties of MSC are inferred to be a specialized function related to their primitive or multipotential nature (26, 30, 31). However, classical literature (32) and recent ontological studies (33) suggest that Fb are among the most primitive cells of adult tissues. In addition, Fb have previously been shown to interact with the immune system as alternative APC, either activating or down-regulating T cells (34–36) and mediating indirect antiproliferative effects on lymphocytes (37, 38). A potent effect of Fb on DC differentiation has also been observed (39). The relationship between the immune interactions of MSC and Fb has not been directly addressed.

The physiological function of MSC-mediated immunosuppression is difficult to resolve. Expansion in vitro and subsequent intravascular injection may create completely artificial interactions with the immune system. Alternatively, it is possible to hypothesize that stromal cells may have fundamental immunomodulatory functions, which in vivo might prevent inappropriate T cell activation or terminate immune responses during regeneration and healing. It is now recognized that T cell activation by APC is conditioned by a number of third parties such as B cells and NK cells (40, 41) and similar regulation by stromal cells would have far-reaching consequences, given their ubiquitous presence at sites of lymphocyte priming and restimulation.
In this study, we aimed to shed light on the phenomenon of MSC-mediated immunosuppression by determining whether generic stromal cells such as dermal Fb were capable of modulating T cell responses in the same fashion. Our findings show that Fb are indeed potent immunoregulatory cells with closely related functional properties. The potential physiological and therapeutic implications are discussed.

Materials and Methods

Cell isolation and culture

Dermal Fb were isolated from 4-mm punch biopsies of skin or from 300-μm dermato ne sections of skin recovered from mammoplasty surgery. Apical dermis was digested for 2–12 h in collagenase (1–2 mg/ml collagenase D; Roche). A single-cell suspension obtained by pipetting, and seeded at 10^6 cells per 25-cm² flask. Passage 0 to passage 1 Fb were obtained by two rounds of adherence to tissue culture plastic and were ≥90% CD73^+CD45^-. MSC were obtained from BM mononuclear cells isolated over Lymphoprep (Fresenius Kabi) according to established protocols (42). Human samples were obtained following informed consent and in accordance with a favorable ethical opinion from North Tyneside Research Ethics Committee. Fb and MSC were passaged in RPMI 1640 with 20% FCS, glutamine, penicillin, and streptomycin (Invitrogen Life Technologies) or Poietics MSC medium with MSC growth supplements, gluta mine, penicillin, and streptomycin (Cambrex). In fibroblast CFU (CFU-F) assays, 10^3 cells from collagenase-digested dermis or 10^7 mononuclear cells from BM aspirate were cultured undisturbed in 25-cm² flasks for 2 wk. Colonies were methanol fixed, stained with Giemsa, and counted manually.

For transdifferentiation assays, passage 1–6 Fb were cultivated in RPMI 1640 with 20% FCS and the following supplements: osteogenic medium, 100 nM dexamethasone (Sigma-Aldrich), and 0.05 mM ascorbic acid (Sigma-Aldrich); chondrogenic medium, 6.25 μg/ml insulin (Sigma-Aldrich), 50 nM ascorbic acid, and 10 ng/ml TGFβ1 (PeproTech); adipogenic medium, 1 μM dexamethasone, 10 μg/ml insulin, 0.5 mM methylisobutylxanthine (Sigma-Aldrich), and 100 μM indomethacin (Sigma-Aldrich). Chondrogenesis was detected by staining for sulfated proteoglycans with 1% Alcian blue in 0.1 N hydrochloric acid, osteoblasts stained for alkaline phosphatase with red violet,
FIGURE 2. Mechanism of suppression of T cell proliferation. A, Fb-mediated suppression of T cell proliferation was observed across Transwell membranes. Control, moDC and T cells in the top chamber; +Fb bottom, dermal Fb separated across the Transwell membrane; +Fb top, dermal Fb cocultured in the top chamber with moDC and T cells. Results show mean ± SEM. Cultures contained the following: 5 × 10^4 moDC, 5 × 10^5 T cells, and 5 × 10^5 Fb. Data shown are one of two experiments performed in entirety; all sections of the experiment were repeated at least six times in total. B, T cell proliferation suppression was also observed using day 4 Fb culture supernatant (+Fb) and Fb stimulated by MLR (+Fb MLR) but not MLR supernatant (+MLR). Supernatants were used at 50:50 with normal medium; *, p < 0.05 with respect to control. Data represent at least three experiments. C, Abrogation of stromal cell suppression of T cell proliferation with blocking Ab to IFN-γ (20 μg/ml). □, minus anti-IFN-γ Ig; ■, plus anti-IFN-γ Ig; ◊, plus isotype control. Cultures contained 10^4 moDC and 10^5 T cells (control) and 10^5 stromal cells where indicated (+Fb; +MSC). Results are representative of at least three experiments. D, Stromal cell suppression of T cell proliferation is unaffected by 20 μM indomethacin. □, minus indomethacin; ■, plus indomethacin. Experimental conditions were as in C. Results are representative of at least four experiments. E, Partial reversal of stromal cell suppression of T cell and adipocytes stained for lipid with Oil Red-O and visualized using laser confocal microscopy.

Monocyte-derived DC (moDC) were generated from magnetically isolated CD14^- monocytes and cultured for 6 days with 50 ng/ml rGM-CSF and IL-4 (R&D Systems) followed by 24-h activation with 1 μg/ml LPS (Sigma-Aldrich), 10 ng/ml IL-1β (PeproTech), and 10 ng/ml TNF-α (PeproTech) as previously described (43). Langerhans cells (LC) and dermal DC were isolated by spontaneous migration from 1 mg/ml dispase (Roche) separated epidermal and dermal sheets cultured with 50 ng/ml GM-CSF over 60 h as previously described (44). CD3^- T cells (purity, >90%) were isolated using Rosette-Sep according to manufacturer’s instructions (StemCell Technologies) and the non tumourigenic keratinocyte cell line, HaCaT, was a gift from Dr. N. E. Fusenig (German Cancer Research Centre, Heidelberg, Germany).

Proliferation assays and cytokine production
T cell proliferation was measured in flat-bottom 96-well plates using a 16-h pulse of 0.548 MBq/ml [3H]thymidine (TRA310; Amersham Biosciences) on day 5 unless otherwise stated. Thymidine incorporation was measured by direct scintillation counting (Matrix 9600; Packard Instrument) or luminescence counter (Microbeta TriLux; PerkinElmer).

Transwell experiments used 0.4-μm pore size inserts in 24-well format (Corning). In restimulation assays, allogeneic moDC and CD3^- T cells were cocultured for 6 days in the presence or absence of dermal Fb orMSC. The nonadherent T cells (79% CD3^- T cells) were harvested and rested for a further 4–6 days in the presence of 0.1 ng/ml IL-2 (Glaxo) before restimulation with the same donor-derived moDC or CD3/CD28-coated beads (1 bead:3 T cells; Dynabeads T cell expander; Invitrogen Life Technologies). Neutralizing anti-IFN-γ Ab (B27) was obtained from BD Biosciences; 1-methyl-l-tryptophan (1-MT) and indomethacin were from Sigma-Aldrich. Supernatants were collected and stored frozen for cytokine assays using the BD Biosciences cytometric bead array (CBA) software, and analyzed with the BD-FCAP Array software, version 1.0.

DC modulation by dermal Fb
A total of 2.5 × 10^5 LPS-, TNF-α-, and IL-1β-matured moDC was cocultured with and without 2.5 × 10^5 dermal Fb in 24-well plates in the presence of 50 ng/ml GM-CSF and IL-4. After 2 days, the mature moDC were washed three times and assessed by flow cytometry. A total of 4 × 10^5 mature moDC (>96% DC) cocultured with and without dermal Fb was stimulated with 4 × 10^5 CD40L-transfected J558L mouse cells line (provided by P. Lane, Birmingham University, Birmingham, U.K.) in 96-well flat-bottom plates for 24 hs. Supernatants were collected and stored frozen for cytokine analysis.

Flow cytometry
Abs were obtained from BD Biosciences unless stated otherwise. Ag (clone), CD3 (Abcam; polyclonal ab5690); CD73 PE (AD2); CD90 FITC (5E10); CD14 PE (M5E2); CD271 FITC (LNGFR) (Miltenyi Biotec; ME20.4-1H4); IDO (Upstate/Millipore; 10.1); GAPDH (Chemicon; 6C5); and prolyl hydroxylase (Abcam; 5B5) were used. Secondary detection was achieved with Alexa 555-conjugated goat anti-rabbit IgG2a and Alexa 633-conjugated goat anti-mouse IgG1 (Invitrogen Life Technologies). Flow cytometry was performed using FACSCalibur (BD Biosciences) and analyzed with FlowJo (Tree Star).

Protein immunostaining
Protein lysates from day 4 Fb (1 × 10^6) from allogeneic reactions (>88% CD45^- Fb) and recombinant human IFN-γ (R&D Systems; 0–1000 IU/ml)-treated culture were separated by gel electrophoresis and electroblotted proliferation by increasing concentrations of 1-MT. ■, Control; ■, +Fb; □, +MSC. Experimental conditions were as in C, p < 0.05 with respect to suppressed cultures with no 1-MT. Results representative of at least five experiments. F, Immunoblots probed with anti-IDO and anti-GAPDH Ab of lysates from Fb cultured under the following conditions. C, Untreated cells; IFN-γ, treated with IFN-γ (1000 IU/ml); MLR -, exposed to MLR alone; MLR +, exposed to MLR plus neutralizing anti IFN-γ Ab; MLR Iso, isotype control. Result from one of two experiments is shown.
onto nitrocellulose filters (Hybond C extra, Amersham Biosciences). The membranes were probed with mouse monoclonal anti-IDO (Upstate/ Millipore; clone 10.1) and anti-GAPDH Abs (Chemicon; clone 6C5), and detected using ECL system (Amersham Biosciences) according to manufacturer’s instructions.

Skin explant assay

A total of 10^6 donor CD3^+ T cells was added to 10^5 recipient mature moDC with and without third-party dermal Fb or MSC and cultured in 24-well plates in 1 ml of RPMI 1640 with 10% FCS for 6 days. CD3^+ T cells (>90% pure) were harvested and rested for a further 4–6 days in the presence of 0.1 ng/ml IL-2. A total of 5 x 10^5 of rested CD3^+ T cells was added to recipient skin obtained from a 4-mm punch biopsy or shave biopsy and cultured for 3 days. Skin sections cultured with CD3^+ T cells were used as background controls. The skin samples were subsequently fixed in formalin, paraffin embedded, and stained with H&E. The skin sections were assessed and scored according to the grading system of Lemere (45) by two independent blinded assessors. The histopathological grading system is as follows: grade 0, normal skin; grade I, mild vacuolar degeneration of basal epidermal layer; grade II, diffuse vacuolar degeneration of basal cells with scattered dyskeratotic bodies; grade III, subepidermal cleft formation; and grade IV, complete epidermal separation.

Microscopy

Phase contrast images were taken with an Olympus inverted microscope fitted with a Pentax digital camera. Bright-field/dark-field images were acquired with a Leica microscope fitted with a Leica imaging system and software. Laser confocal microscopy was performed using Leica TCS SP2 UV confocal microscope and analyzed using LCS V2.51 imaging software (Leica).

Statistical analyses

Unpaired t test and ANOVA for parametric and Mann-Whitney U test and Kruskal-Wallis test for nonparametric data were performed using Prism 4.0 (GraphPad Software). All p values are two-tailed.

Results

Fb suppress allogeneic T cell proliferation

Dermal Fb were found to be potent suppressors of allogeneic T cell responses to mature moDC. Suppression of proliferation was observed at cell ratios of 1 Fb to 10 T cells, comparable with the effect of BM MSC (Fig. 1A) and occurred using passaged stromal cells that were third party to both T cells and DC. We also observed that allogeneic T cell responses to epidermal LC and dermal DC were inhibited by freshly isolated dermal Fb from the same skin sample (Fig. 1B). The finding that fresh Fb suppress T cell proliferation primed by cutaneous DC from the same skin sample with equal potency as passaged Fb demonstrates that immunosuppression is not a property restricted to rare subpopulations of stromal cells that only expand in vitro. Irradiation, which rendered Fb vegetative, did not abrogate suppression (Fig. 1C), and Fb did not merely skew the kinetics of T cell stimulation (Fig. 1D). Other components of the skin, such as keratinocytes, did not suppress T cell proliferation at similar cell ratios (Fig. 1E). The specificity and potency of Fb-mediated suppression in vitro is hard to put in a physiological context. Examination of the frequency of Fb, T cells, and APC in dermis by collagenase digestion and flow cytometry showed an in situ ratio of 43.2:21.5:12.5 (Fig. 1F), i.e., a Fb:T cell ratio of 2:1, greatly in excess of that used in the experiments described. Confocal microscopy also confirmed that Fb were closely apposed to dermal T cells and DC in situ (Fig. 1G).

T cells induce IFN-γ-dependent IDO expression in Fb

T cell proliferation was inhibited across a Transwell membrane by stromal cells in the lower chamber and APC and T cells in the upper chamber, although coculture frequently caused more potent suppression (Fig. 2A). This result shows that soluble factors mediate at least part of the suppressive effect but that close contact either favors short-acting soluble mediators or provides an additional
direct signal. Supernatants from Fb/MLR cocultures suppressed T cell proliferation to a similar extent to Transwell experiments and much more than Fb monoculture supernatant (Fig. 2B). Together, these results suggest that stimulated T cells induce Fb to elaborate inhibitory mediators, creating a negative-feedback loop. Prompted by previous work using MSC (6, 12), we investigated the role of IFN-γ and found that blocking Abs to IFN-γ completely reversed the suppressive effect of Fb and MSC, compared with no treatment or isotype control (Fig. 2). These results are in keeping with a direct action of Fb on T cells rather than through modulation of mature APC, at least under these experimental conditions.

**Fb do not modulate mature moDC**

Stromal cells are known to alter the differentiation and maturation of DC from monocytes (10, 11, 39), and we investigated whether Fb modulated the activation and cytokine secretion of mature moDC used in these experiments. Expression of CD80, CD83, CD86, and HLA-DR was not significantly altered by coculture with Fb (Fig. 3A) nor was release of IL-10, IL-12, TNF-α, and IFN-γ (Fig. 3B). These results are in keeping with a direct action of Fb on T cells rather than through modulation of mature APC, at least under these experimental conditions.

**Suppression of T cell proliferation is reversible**

Suppression of T cell proliferation was observed when T cells were stimulated with CD3/CD28-coated beads or moDC, again consistent with a direct effect of Fb on T cells. Much higher rates of DNA synthesis were recorded with CD3/CD28-coated beads but relative inhibition was comparable (Fig. 3C). Previous reports have shown T cell anergy after exposure to murine MSC (7). In our hands, CD3/CD28-coated beads stimulated vigorous proliferation comparable with controls, when T cells were rested for 4–6 days after initial sensitization in the presence of Fb or MSC (Fig. 3D). The reversible proliferation of T cells suppressed by stromal cells was in marked contrast to the profound hyporesponsiveness of T cells primed directly by suboptimal or immature APC such as freshly isolated CD14+ peripheral blood monocytes (without Fb).

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Table I. *Modulation of T cell phenotype by Fb and MSCa*

<table>
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<tr>
<th></th>
<th>Expt. 1</th>
<th>Expt. 2</th>
<th>Expt. 3</th>
<th>Expt. 4</th>
<th>Mean Fold Induction</th>
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<td>IFN-γ (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Control</td>
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<td>8,491</td>
<td>20,292</td>
<td>23,613</td>
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<tr>
<td>+Fb</td>
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<td>9,312</td>
<td>28,221</td>
<td>24,068</td>
<td>1.18</td>
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<tr>
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<td>9,793</td>
<td>28,917</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
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<td>433</td>
<td>1,945</td>
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<td>+Fb</td>
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<td>883</td>
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<td>1,142</td>
<td>3,385</td>
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<td>IL-10 (pg/ml)</td>
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<td></td>
<td></td>
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<tr>
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<td>65</td>
<td>34</td>
<td>12</td>
<td>83</td>
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<tr>
<td>+Fb</td>
<td>494</td>
<td>192</td>
<td>25</td>
<td>128</td>
<td>4.32*</td>
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<td>408</td>
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<td>384</td>
<td>493</td>
<td>424</td>
<td>5.84*</td>
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</table>

* Cytokine secretion by restimulated T cells of the experiment described in Fig. 3D. In the left column, Control, +Fb, and +MSC refer to the conditions of primary sensitization prior to resting and restimulation of T cells. Supernatants were analyzed on day 3. 
* p < 0.05 using ANOVA with a statistically significant positive trend comparing control, +Fb, and +MSC for IL-10 and IL-4, respectively.

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**FIGURE 4.** Inhibition of in vitro graft vs host (GVH) reaction by stromal cells. A, Histological appearance and GVH reaction pathological grading of skin explants according to the Lerner scoring system (45). Control, T cells only; TmoDC, T cells primed by moDC; TmoDC + Fb, T cells primed by moDC in the presence of Fb; TmoDC + MSC, T cells primed by moDC in the presence of MSC. T cells primed by mature moDC (TmoDC) do not react to third party skin. B, Summary of pathological grades from five independent experiments, performed in duplicate, showing individual data points. p < 0.05 using the Kruskal-Wallis test comparing the three groups: TmoDC, TmoDC + Fb, and TmoDC + MSC. T cells were prepared for the assay by an initial sensitization using moDC derived from the skin donor with or without stromal cells. After a 4- to 6-day rest period with 0.1 ng/ml IL-2, sensitized T cells were incubated with skin for 72 h. T cells alone (control) or T cells sensitized with moDC (TmoDC) or moDC in the presence of Fb (TmoDC + Fb) or MSC (TmoDC + MSC) were used as indicated. Skin sections were fixed in formalin and stained with H&E before analysis.
Reprogramming of T cell cytokines by stromal cell conditioning

Although T cells previously exposed to stromal cells were able to proliferate normally on restimulation, we investigated whether there had been additional functional consequences such as the modulation of cytokine production. In these experiments, T cells were removed from the initial coculture with stromal cells, rested and then restimulated with CD3/CD28-coated beads in the absence of stromal cells. We were surprised to discover that there was marked induction of Th2 cytokines, IL-4 and IL-10, in cells exposed to stromal cells, but no increase in Th1 cytokines, IFN-γ and TNF-α (Table I). MSC appeared more potent in this regard, inducing ~2-fold greater levels of Th2 cytokines than Fb.

In contrast, there was little evidence of cytokine modulation by stromal cells during the initial sensitization phase, although it was notable that high levels of IFN-γ were produced in all cultures in keeping with the IFN-γ dependence of suppression described above (data not shown).

Amelioration of graft-vs-host reaction by exposure to stromal cells

Although MSC are reported to ameliorate clinical GVHD, it is difficult to correlate these observations directly with immunomodulatory effects measured in vitro. In an attempt to resolve this, we adapted an in vitro model of human GVHD (46, 47) to determine whether there was any association between the modulation of cytokine production by stromal cells and histopathological reactions in human GVHD target tissue. This model would allow detection of direct host tissue damage upon host Ag-specific restimulation of stromal cell modulated-donor T cells to assess potential therapeutic effect. In this experiment, a skin fragment is exposed for 72 h to allogeneic T cells previously sensitized by moDC from the skin donor. The resulting pathological reaction in the skin is then graded according to the Lerner system (Fig. 4A) (45). This model has been used in mechanistic studies or prediction of human clinical GVHD (47, 48). T cells stimulated with mismatched skin...
Table II. Clonogenic potential of Fb and MSC*

<table>
<thead>
<tr>
<th></th>
<th>BM Mononuclear Cells</th>
<th>Digested Dermal Cells</th>
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<tbody>
<tr>
<td></td>
<td>Expt.1</td>
<td>Expt.2</td>
</tr>
<tr>
<td>CFU-F/cell</td>
<td>0.000014</td>
<td>0.000015</td>
</tr>
<tr>
<td>CD45⁻CD73⁺ cell</td>
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<td>0.000005</td>
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<tr>
<td>CFU-F/CD45⁻ CD73⁺</td>
<td>0.23</td>
<td>0.30</td>
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</table>

* Estimates of the clonogenic potential (the ratio of CFU-F to CD73⁻CD45⁺ cells) from individual specimens of BM mononuclear cells compared with digested dermis.

**Discussion**

In this study, we have attempted to address whether MSC-mediated immunosuppression is shared by dermal Fb, an easily accessible and alternative stromal cell. In addition to demonstrating a similar magnitude of suppressive effects, we have also provided evidence that there are many mechanistic similarities between Fb and MSC using assays previously reported for MSC and presented new data on the modulation of cytokines and amelioration of in vitro GVHD by both types of cell. We have presented a phenotypic description of dermal Fb and have attempted to shed some light on the possible physiological relevance of stromal cell-mediated immunosuppression.

Our results show that dermal Fb inhibit T cell proliferation in vitro in response to allogeneic moDC with similar potency to BM-derived MSC. In addition, freshly isolated Fb inhibit alloreresponses to autogenous cutaneous APC at cell ratios that are consistent with the frequency of Fb, T cells, and APC occurring in situ. Suppression was not observed with use of a nonstromal cell line. Previous studies have shown that Fb are not immunologically inert (34–36). More recently, they have been shown to regulate T cell development (51, 52) and survival (53). Immunosuppressive effects of Fb have been observed in response to mitogens and other stimuli (37, 38). We have now shown that these properties relate very closely to the phenomenon of MSC-mediated T cell suppression in vitro. Although we did not see any modulation of APC function by Fb using mature moDC, it is known that Fb and MSC are able to influence the earlier stages of DC differentiation in other systems (10, 11, 39, 54, 55). Together, these data suggest that stromal cells have pleiotropic immunoregulatory functions.

Our studies suggest that Fb use similar mechanisms to those reported previously for MSC to suppress T cell proliferation. Using Transwells and conditioned supernatants, we found evidence for soluble mediators. Similar results have been reported using MSC (12, 16, 56–58), although there is some variation due to different experimental designs. Transwell experiments with stromal cells in the lower chamber and stimulated T cells in the smaller upper chamber result in more pronounced suppression than the converse, which has led some investigators to conclude that contact effects are paramount (5, 10). We do not dispute that proximity augments the suppressive effect, because the greatest inhibition is always seen within cocultures, but true contact-mediated suppression has not been demonstrated in the literature. Failure to test MSC-stimulated T cell coculture medium for suppressive effects (rather than MSC-conditioned medium alone) has also led some investigators to conclude that soluble mediators are not produced by MSC (5, 10). Indeed, inhibition by conditioned medium from Fb or stimulated T cells alone is much less potent than from Fb cocultured with stimulated T cells. Stromal cells appear to require induction by activated T cells to mediate their effects. This is supported by our observation that neutralizing Abs to IFN-γ abrogate Fb-mediated suppression, as has been observed in MSC (12).

A role for IFN-γ in promoting immunosuppression mediated by stromal cells has been suspected for some time (37, 38) and appears to form the efferent limb of a negative-feedback loop between T cells and stroma. Induction of IDO and the acceleration of tryptophan degradation may be at least one mechanism of the afferent limb. In common with MSC studies (6, 12), we have shown that IFN-γ induces IDO in Fb and that suppression is at least partly reversed by the addition of l-tryptophan. The lack of complete reversal with this maneuver is likely to be explained by the continued accumulation of tryptophan metabolites kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid, which suppress T cell proliferation, although we did not formally test this (59). Supporting the relevance of this mechanism in vivo, up-regulated expression of IFN-γ, IDO, and increased tryptophan degradation have been found in psoriatic skin lesions (60) and in the intrinsic resistance of Fb to Toxoplasma gondii infection (61). We did not find any evidence for the role of PG in T cell-suppressive effects...
by dermal Fb or BM MSC. PG synthesis and action has been recorded previously using FBMC rather than purified T cells mixed with MSC in a shorter 3-day coculture (9) and in mitogen rather than allostimulation of T cells (62).

In common with several reports using MSC (4, 58), we found that Fb-mediated suppression of T cells was reversible, in contrast to direct allostimulation using immature population of APC, such as peripheral blood monocytes (without Fb). Priming with monocytes alone induces anergy due to TCR ligation in the absence of adequate costimulation, as previously described (63). A murine study also described anergy but using a shorter 48-h exposure to MSC (7). In addition to suppression of T cell proliferation, previous reports have examined the potential of MSC to modify cytokine secretion (7, 9, 10, 58). These have usually examined the primary exposure to MSC and demonstrated variable modulation of IFN-γ, IL-10, and TNF-α (7, 10, 58). One study also demonstrated enhanced skewing toward Th2 cytokine production when MSC were added to Th2-promoting culture conditions (9). Our results are the first to show a reprogramming of T cell cytokine production after transient exposure to both MSC and Fb, but whether the in vitro efficacy of MSC can also be emulated by Fb remains undetermined. Because sufficient Fb for cellular therapy can be grown from a single punch biopsy in 3 wk, the opportunity to use autologous or directed donor therapy without recourse to more invasive procedures such as BM aspiration warrants further exploration.

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Disclosures

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