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Type 2 Helper T-Cell Predominance and High CD30 Expression in Systemic Sclerosis

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The pattern of cytokine production of skin-infiltrating T cells from patients with progressive systemic sclerosis was investigated. Most CD4$^+$ T-cell clones generated from skin biopsy specimens showed a type 2 helper (Th2) cytokine profile (production of interleukin-4, but no interferon (IFN)-γ). High interleukin-4 but little or no IFN-γ mRNA expression was found by *in situ* hybridization in skin perivascular mononuclear cell infiltrates. The immunohistochemical analysis revealed CD30 expression by high numbers of CD4$^+$ T cells in the same specimens. Finally, the great majority of patients with diffuse disease had elevated levels of soluble CD30 in their sera. These data suggest the existence in patients with progressive systemic sclerosis of a predominant activation of Th2-like T cells, which may account for the major alterations (endothelial cell injury, fibrosis, and autoantibody production) occurring in this disease. *(Am J Pathol 1997, 151:1751–1758)*

Progressive systemic sclerosis (SSc) is a disorder characterized by inflammatory, vascular, and fibrotic changes of the skin (scleroderma) and a variety of internal organs, most notably the gastrointestinal tract, lungs, heart, and kidney. In the skin, a thin epidermis overlies compact bundles of collagen, which lie parallel to the epidermis. Increased numbers of T cells may be present at the border of skin lesions as well as in other organs in the early stages of the disease.[1] Both CD4$^+$ and CD8$^+$ T cells are found in the skin of patients with progressive SSc, but CD4$^+$ T cells are the major subpopulation.[1,2] Furthermore, cellular autoimmunity to collagen and laminin[3] and the development of scleroderma-like lesions in patients with graft-*versus*-host disease (GVHD)[4] also suggest a role for activated T cells in the pathogenesis of SSc. Several cytokines secreted by T cells or other cells of the immune system may contribute to modulate fibrosis and promote vascular activation/damage in SSc. Peripheral blood mononuclear cells from SSc patients produce soluble factors *in vitro* that stimulate fibroblast proliferation, protein synthesis, and collagen production to a greater degree than do lymphocytes from controls.[5] Cytokines capable of altering endothelial cell function have been found in SSc sera or tissues, including interleukin (IL)-1, IL-2, IL-4, IL-6, IL-8, tumor necrosis factor, transforming growth factor-β, and platelet-derived growth factor.[6–9]

Recent advances in our understanding of the immune response have identified subpopulations of CD4$^+$ helper T cells (Th cells), termed Th1 and Th2, by their ability to release different sets of cytokines, which can be shown to be associated with different patterns of immunological reaction. Th1 cells produce IFN-γ and tumor necrosis factor-β and are responsible for phagocyte-dependent host responses characterized by delayed-type hypersensitivity reaction, or granulomatous pattern, which are involved in the protection against intracellular parasites. Th2 cells produce IL-4 and IL-5 and are responsible for phagocyte-independent, antibody-mediated responses that are implicated in the protection against gastrointestinal nematodes and in allergic conditions.[10,11] Activated Th2 cells also consistently and persistently express CD30,[12–14] a member of the tumor necrosis factor-receptor superfamily,[15] and release measurable amounts of soluble CD30 (sCD30), both *in vitro* and *in vivo*.[12,14,16]

The aim of the present study was to examine the profile of cytokine production as well as the expression of CD30 by skin-infiltrating T cells from patients with SSc. The hypothesis tested was that activated T cells in SSc patients mainly belong to the Th2 subset whose pattern of cytokines may determine, at least in part, the nature of the inflammatory response seen in SSc.

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Materials and Methods

Subjects

Skin biopsy specimens for T-cell cloning were obtained from two SSc patients and three patients with atopic dermatitis (AD). Skin biopsy specimens for immunohistochemical and/or *in situ* hybridization analyses were obtained from three patients with SSc, three patients with AD, one patient with GVHD, one patient with alopecia areata, and one patient with contact dermatitis. All SSc patients used through the study had low duration (<3 years) and active untreated disease. Blood samples for the measurement of sCD30 were taken from 43 patients with SSc (35 women and 8 men, aged 20 to 60 years) and from 23 age- and sex-matched control subjects. The procedures followed in the study were in accordance with the ethical standards of the responsible regional committee on human experimentation.

Generation of T-Cell Clones

Specimens of skin biopsies from both SSc patients and controls were disrupted in small fragments (2-mm diameter). Single fragments were cultured for 7 to 10 days in 24-well plates (Costar, Cambridge, MA) in 2 ml of RPMI 1640 medium (Seromed, Berlin, Germany) supplemented with 2 mmol/L L-Glutamine (Life Technologies, Grand Island, NY), 2×10^{-5} 2-mercaptoethanol, nonessential amino acids (Life Technologies) and sodium piruvate (Life Technologies) (complete medium), 10% fetal calf serum (HyClone, Logan, UT), and 20 U/ml recombinant IL-2.[17,18] After 7 to 10 days, T-cell blasts were cloned under limiting dilution conditions (0.3 cells/well) in 96 round-bottomed microwell plates (Nunclon, Nunc, Denmark), containing 10^5 irradiated (6000 rad) allogeneic peripheral blood mononuclear cells as feeder cells, 0.5% (v/v) phytohemagglutinin (Life Technologies, Grand Island, NY), and recombinant IL-2 (rIL-2, 20 U/ml; Eurocetus, Milan, Italy), as detailed elsewhere.[17,18] Growing microcultures were then expanded at weekly intervals with 20 U/ml rIL-2 and 10^5 irradiated feeder cells. Cell surface marker analysis of T-cell clones was performed by using fluorescein isothiocyanate- and phycoeritrine-conjugated anti-CD4 and anti-CD8 mAbs (Becton Dickinson, Mountain View, CA) on a Cytoron absolute cytofluorimeter (Ortho Pharmaceuticals, Raritan, NJ).

Production of Cytokines by T-Cell Clones

The ability of T-cell clones to produce cytokines was evaluated after 36 hours of stimulation of 10^6/ml T-cell blasts with phorbol-12-myristate 13-acetate (Sigma, St. Louis, MO; 20 ng/ml) and anti-CD3 mAb (Ortho Pharmaceuticals; 100 ng/ml), as previously described.[18,19] Cell-free supernatants were assayed for IFN-γ and IL-4 content by enzyme-linked immunosorbent assay (ELISA) systems. The quantitative determination of IFN-γ was performed by a commercial system (BioSource International, Camarillo, CA). For the measurement of IL-4 an in-house-made ELISA using commercial mAb (PharMingen, San Diego, CA) was used.[19] Cytokine levels 5 SD more than the mean levels of control supernatants (derived from irradiated feeder cells alone) were regarded as positive. T-cell clones able to produce IFN-γ but no IL-4 were categorized as Th1-like; clones able to produce IL-4 but no IFN-γ were categorized as Th2-like; clones producing both IL-4 and IFN-γ were categorized as Th0-like.

In Situ *Hybridization*

In situ hybridization was performed on frozen skin sections, which were mounted onto gelatin-coated slides and fixed with 4% paraformaldehyde for 20 minutes at room temperature. Sections were subsequently treated with 0.2 N HCl for 20 minutes, pronase (0.125 mg/ml) for 10 minutes, O.1 mol/L glycine for 30 seconds, and 4% paraformaldehyde for 20 minutes. Then, sections were rinsed with phosphate-buffered saline, acetylated, and dehydrated in increasing ethanol concentrations. Thirty μl of the hybridization solution (40% formamide, 4× SSC, 10 mmol/L dithiothreitol, 1× Denhardt's solution, 10% dextran sulfate, 0.1 mg/ml sheared herring sperm DNA, and 1 mg/ml yeast tRNA) containing 8×10^5 cpm of ^{35}S-labeled human IL-4 or IFN-γ RNA antisense probe were applied to each section and covered with parafilm. Hybridization was carried out at 52°C for 16 hours. Removal of the nonspecifically bound probe by RNase digestion and autoradiography were performed as described.[20] Sections were subsequently counterstained with hematoxylin-eosin-phloxine and mounted with Permount. An average of two sections were analyzed for each tissue sample. Negative controls consisted of hybridization to a sense RNA probe. Both sense and antisense probes were synthesized from a IL-4 cDNA and subcloned in a pGEM-T plasmid vector (Promega, Madison, WI). The plasmid containing the IL-4 330-bp cDNA was subsequently linearized with *Sal*I or SPH I restriction enzymes and the plasmid containing the 427-bp cDNA was linearized with *Nco*I or *Pst*I restriction enzymes, followed by phenol-chloroform extraction and ethanol precipitation. Thereafter, sense and antisense RNA radiolabeled probes were synthesized using SP6 or T7 RNA polymerases (Riboprobe Gemini System, Promega) in the presence of [^{35}S]α-thio-UTP (1300 mCi/mmol; NEN Life Science Products, Paris, France).

Immunohistochemistry

Immunohistochemical staining was performed on 10-μm cryostat sections fixed in 4% paraformaldehyde for 20 minutes. Sections were subsequently exposed to 0.3% hydrogen peroxide-methanol solution to quench endogenous peroxidase activity. After a 20-minute preincubation with normal horse serum (Vectastain ABC kit; Vector Laboratories, Milan, Italy), sections were layered for 30 minutes with anti-CD3 (Ancell, Bayport, MN; 5 μg/ml), anti-CD4 (Ancell, 5 μg/ml), anti-CD30 (Immunotech, Marseille, France; 4 μg/ml), anti-IFN-γ (5 μg/ml), and anti-

IL-4 (5 μg/ml; kindly provided by C.H. Heusser, Novartis, Basel, Switzerland) murine mAbs, followed by biotinylated anti-mouse IgG antibody and the avidin-biotin-peroxidase complex (Vectastain ABC kit, Vector Laboratories), as described.[21] A peroxidase substrate used was 3-amino-9-ethylcarbazole (Sigma). Finally, sections were counterstained with Gill's hematoxylin and mounted with Kaiser's gelatin. All incubations were performed at room temperature. As negative control, primary mAb was omitted or replaced with an isotype-matched antibody with irrelevant specificity. The number of positive cells was determined from counts performed on two fields (×400) randomly selected from every biopsy specimen in a blinded fashion.

Measurement of sCD30 in the Serum

Serum levels of sCD30 were determined by the commercial sandwich ELISA Dako CD30 (Dako, Gastrup, Denmark) based on the use of two mAbs reactive with two different epitopes of the 88-kd sCD30 molecule, as described.[22]

Results

Th2-Like Profile of T Cell Clones Generated from the Skin of SSc Patients

Parallel biopsy specimens of skin tissue from two patients with SSc and three patients with AD were cultured for 7 to 10 days in IL-2-conditioned medium to preferentially expand the *in vivo* activated T cells present in the specimens. T-cell blasts were recovered and cloned by limiting dilution according to a high efficiency protocol using phytohemagglutinin-stimulation in the presence of irradiated feeder cells and IL-2.[18] A total number of 97 and 153 clones were generated from the skin of SSc and AD patients, respectively. One hundred seventeen T-cell clones obtained from the skin specimens of AD were $CD4^+$, the other 36 being $CD8^+$. In contrast, virtually all T-cell clones generated from the skin of SSc patients were $CD4^+$ (96 out of 97), only one being $CD8^+$. To characterize the cytokine profile of T-cell clones, 10^6 T-cell blasts from each clone were stimulated for 36 hours with phorbol 12-myristate 13-acetate plus anti-CD3 mAb, and cytokine (IL-4, IFN-γ) concentrations were measured into cell-free culture supernatants. The results of these experiments are summarized in Figure 1. The majority of $CD4^+$ T-cell clones from AD patients produced both IFN-γ and IL-4, thus showing a mixed cytokine profile (Th0-like), a few producing IFN-γ alone (Th1-like), or IL-4 alone (Th2-like). By contrast, the great majority of $CD4^+$ T-cell clones from SSc patients produced IL-4 but no IFN-γ, which is a restricted Th2-like profile and the other being Th0-like.

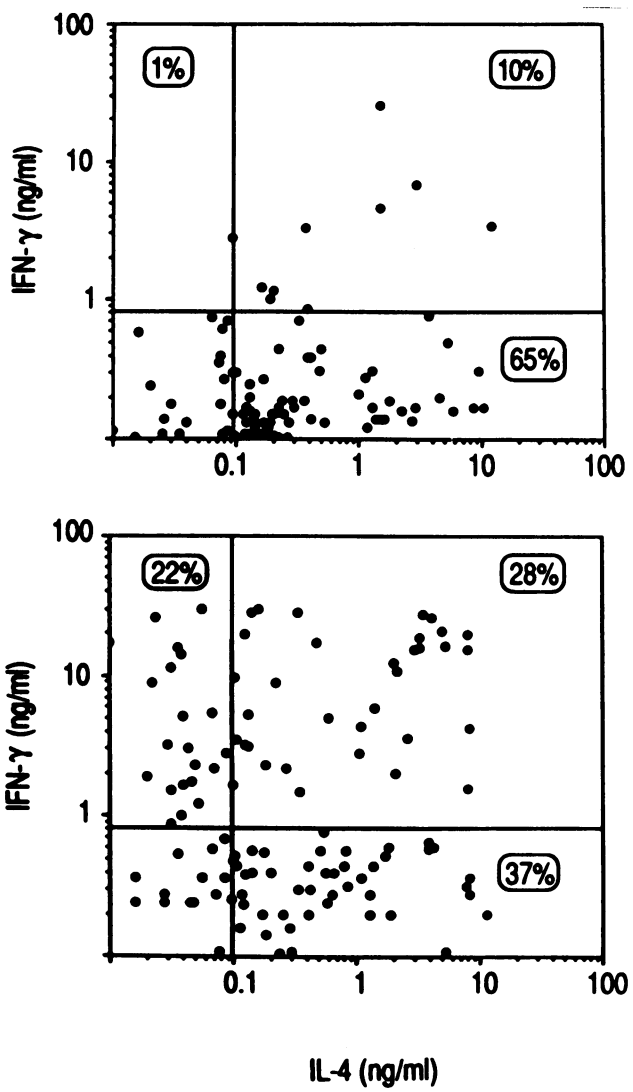

Figure 1. Restricted Th2-like profile of cytokine production by $CD4^+$ T-cell clones generated from skin biopsy specimens of SSc patients. T-cell clones were generated from the skin biopsy specimens of two patients with SSc (**top**) and three patients with AD (**bottom**) by culturing skin fragments for 7 days in IL-2-supplemented medium, followed by stimulation of single T cells with phytohemagglutinin and IL-2 in the presence of irradiated feeder cells. 10^6 T-cell blasts from each clone were stimulated for 24 hours with phorbol 12-myristate 13-acetate plus anti-CD3 mAb and cytokines released into cell-free supernatants measured by appropriate ELISAs. **Lines** represent cut off values (IFN-γ, 0.8 ng/ml; IL-4, 0.1 ng/ml), calculated as 5 SD more than values found in cultures containing feeder cells alone.

IL-4 and CD30 Reactivity by Skin-Infiltrating $CD4^+$ T Cells from SSc Patients

To ascertain whether the Th2 predominance found at clonal level in the skin of SSc patients really reflects the nature of the immune response occurring *in vivo*, skin biopsy specimens from three SSc patients were assessed by *in situ* hybridization for the expression of IL-4 and IFN-γ mRNA. High IL-4 but little or no IFN-γ mRNA expression was found in skin mononuclear cell infiltrates of all three SSc patients tested (Figure 2). The skin biopsy specimens from the three SSc patients and from six controls (three patients with AD, one patient with GVHD, one patient with alopecia areata, and one patient with contact dermatitis) were also assessed

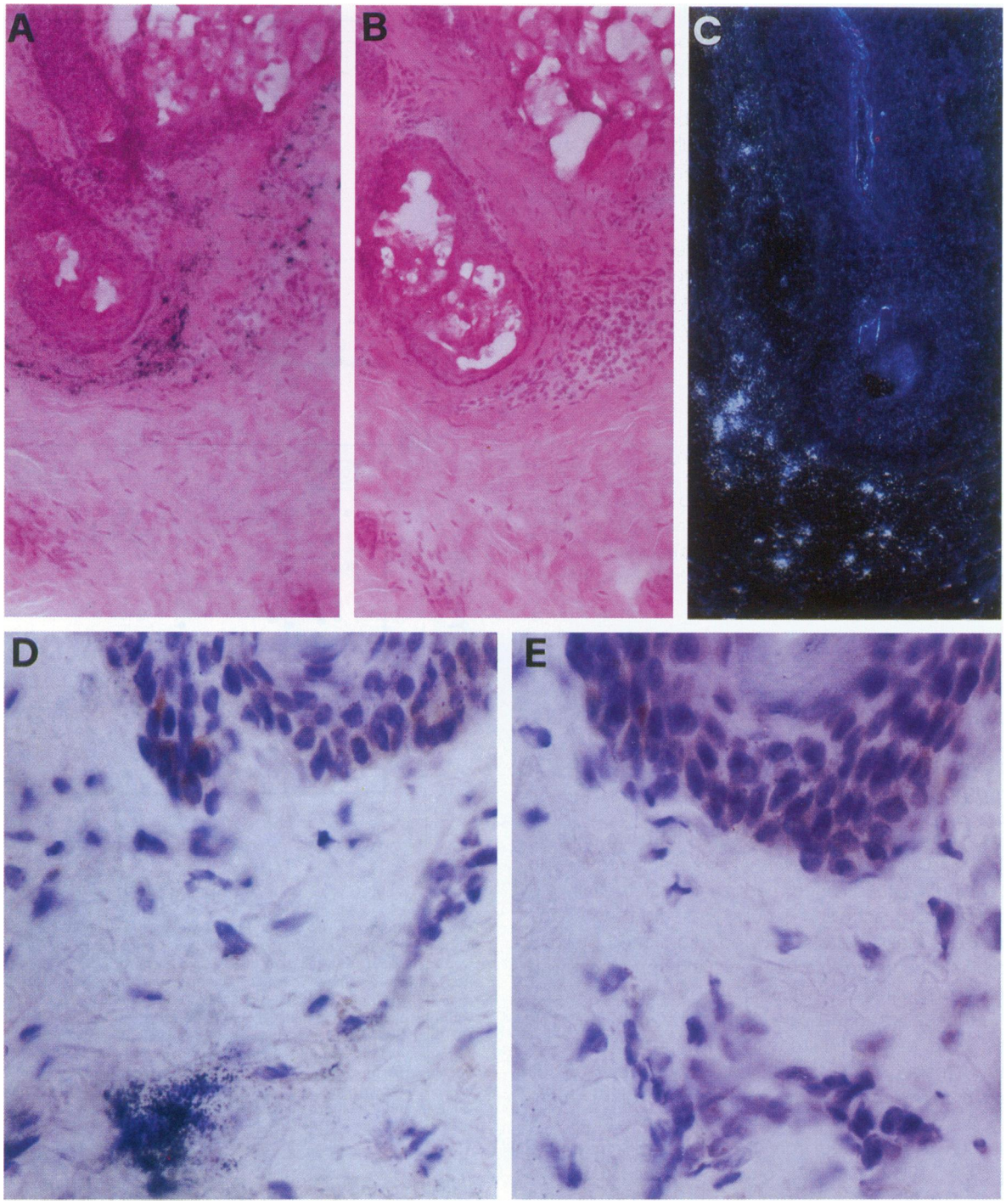

Figure 2. IL-4 mRNA expression in dermal perivascular infiltrates from SSc patients. **A:** IL-4 mRNA expression (**black signal**) in a skin biopsy section taken from a SSc patient and hybridized with labeled antisense IL-4 probe (magnification, ×100). **B:** Lack of signal in an adjacent section hybridized with labeled antisense IFN-γ probe (magnification; ×100). **C:** IL-4 mRNA expression (**white spots**) around a piliferous follicle in another SSc patient (**dark field**, magnification, ×100). **D:** IL-4 mRNA expression (**black spots**) in the skin of a third SSc patient (magnification, ×400). **E:** Lack of IFN-γ mRNA expression in an adjacent section.

by immunohistochemistry for the expression of CD3, CD4, IL-4, IFN-γ, and CD30. In all cases, high numbers of skin-infiltrating mononuclear cells stained positive for both CD3 and CD4. IFN-γ-expressing cells were not detected by immunohistochemistry in the skin of any of SSc patients, but noticeable numbers of IFN-γ-expressing cells were found in the skin of patients with alopecia areata (20% of $CD4^+$ T cells) or contact dermatitis (6% of $CD4^+$ T cells). By contrast, IL-4-expressing cells were never detected by immunohistochemistry in the skin of any patient examined. However, in the skin of all three SSc patients remarkable numbers of $CD4^+$ cells stained positive for CD30 (59, 81, and 87%, respectively). High numbers of $CD4^+$ T cells showing CD30 positivity (57%) were also found in the skin of the patient with GVHD, whereas much lower numbers of $CD4^+$ T cells stained for CD30 in the skin of patients with AD

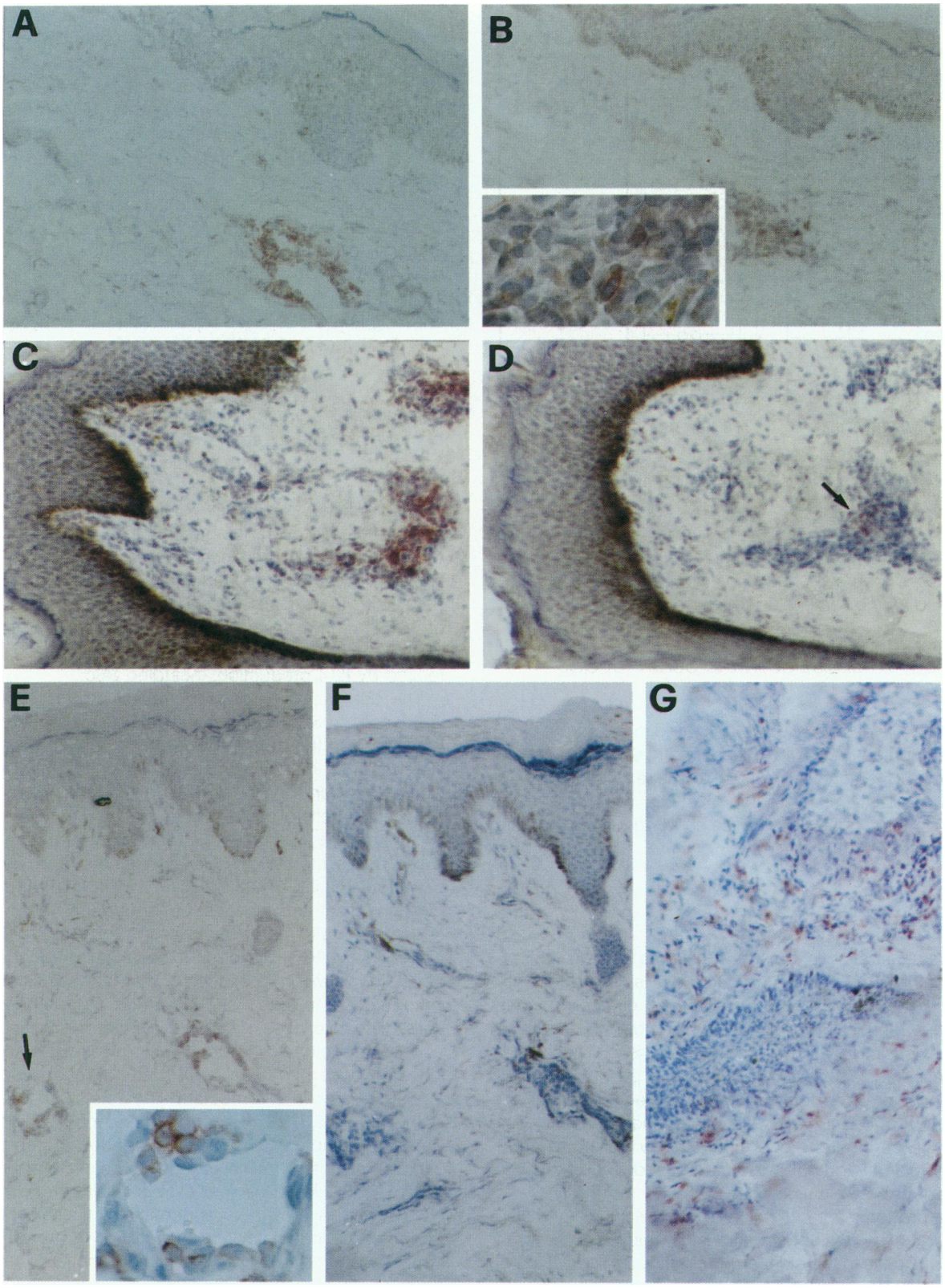

Figure 3. CD30 expression by $CD4^+$ T cells in dermal perivascular infiltrates from SSc patients. **A:** Intense immunostaining (**red color**) for CD4 is present over almost every cell infiltrating the derma of the SSc patient (magnification, ×100). **B:** Staining for CD30 in an adjacent section (magnification ×100); in the **inset**, $CD30^+$ T cells at higher magnification (magnification, ×400). **C:** Intense CD4 immunostaining in cells infiltrating the derma of one patient with AD (magnification, ×100). **D:** Staining for CD30 in a section adjacent to **C** where a few cells, as indicated by the **arrow**, are immunoreactive. **E:** $CD30^+$ T cells in the perivascular dermal infiltrates of another patient with SSc (magnification, ×100); in the inset, $CD30^+$ T cells at higher magnification (magnification, ×400). **F:** Lack of staining for IFN-γ in an adjacent section (magnification, ×100). **G:** IFN-γ immunoreactivity in the skin biopsy specimen from a patient with alopecia areata after staining with the same anti-IFN-γ antibody (magnification, ×100).

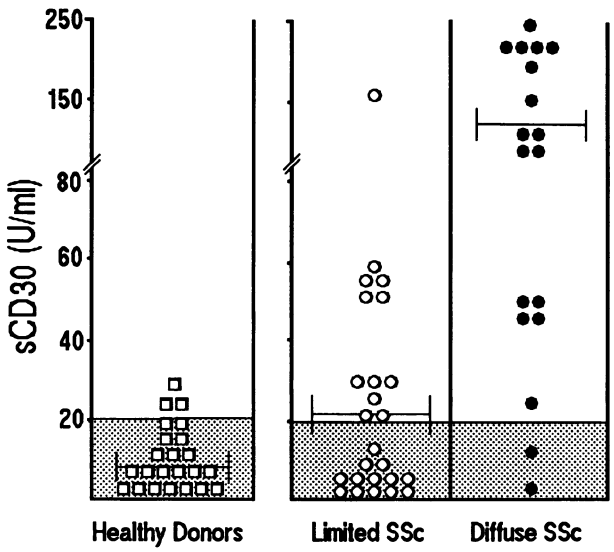

Figure 4. Elevated levels of sCD30 in the serum of patients with SSc. Serum levels of sCD30 were assessed by sandwich ELISA, as reported in Materials and Methods.

(6, 9, and 11%), alopecia areata (23%), or contact dermatitis (10%). Figure 3 shows some representative pictures obtained by the immunohistochemical analysis.

High Levels of sCD30 in the Serum of SSc Patients

Serum levels of sCD30 were measured in the serum of 23 normal subjects and 43 patients with SSc, 25 showed limited and 18 showed diffuse disease. High levels of sCD30 (>20 U/ml) were found in the majority of SSc patients and virtually in all those showing diffuse disease (Figure 4).

Discussion

The demonstration in the skin of patients with SSc of IL-4, but not IFN-γ, mRNA and of $CD4^+$ T cells developing into IL-4-, but not IFN-γ-, producing T-cell clones provides strong evidence for a Th2-like pattern of cytokine gene expression and cytokine production in SSc. These findings are consistent with the results of previous studies showing increased IL-4 production by activated peripheral blood mononuclear cells,[6] increased levels of IL-4 mRNA in peripheral blood mononuclear cells and bronchoalveolar lavage cells,[7] and increased levels of IL-4 in the serum of SSc patients.[8,9]

The predominant activation of Th2 cells in SSc is also supported by the observations of high numbers of $CD30^+$ T cells in the skin, and of high levels of sCD30 in the serum from patients with SSc. Expression of CD30 has indeed been found to be associated with Th2-like responses both *in vitro* and *in vivo*.[12–14,16,23] By contrast, no $CD30^+$ T cells were found either in the gastric antrum of *Helicobacter pilori*-infected patients or in the gut of patients with Crohn's disease in which activated T cells showing a Th1 profile of cytokine production predominate.[24,25] In the present study, high numbers of $CD30^+$ T cells were found in the skin of one patient with GVHD, which is also considered as a Th2-dominated disorder.[26,27] Of note is that the numbers of $CD30^+$ T cells in the skin of both SSc and GVHD patients were higher than those observed in the skin of patients with AD, an inflammatory skin disorder, which is probably triggered by the activation of allergen-reactive Th2-like cells.[28]

The reason for the preferential association between CD30 and Th2-dominated responses has recently been clarified. In both mice and humans, CD30 expression has been found to be dependent on IL-4 production.[29,30] Therefore, in contrast to the results of a previous report showing that CD30 does not discriminate between Th1- and Th2-type T cells,[31] its expression appears to be restricted to fully differentiated Th2 cells and to other activated T cells that behave as Th2 precursors, whereas committed Th1 effectors do not express CD30.[29,30] Thus, because IL-4 generated by activated T cells is rapidly transported and therefore usually does not accumulate in sufficient concentrations to be easily detected by immunohistochemical methods[32] (as in the present study), *in vivo* detection of high numbers of CD30-expressing cells can represent an excellent surrogate marker for the identification of immune responses dominated by high and persistent IL-4 production. Moreover, the measurement of sCD30 serum levels might provide a useful tool for monitoring the severity and activity of disorders characterized by IL-4 production, including SSc.

The demonstration of predominant activation of Th2-like T cells in SSc provides new insights into the pathophysiology of the disease. One of the earliest abnormalities of SSc is a collapse of vimentin intermediate filaments around the nucleus of endothelial cells. Next, activated T cells migrate into the skin where they scatter throughout the subcutaneous tissue and dermis or localize to areas around the vessels, nerves, and skin appendages. Coincidental with T-cell infiltration, additional morphological and functional changes are noted in the endothelial cells, and tissue fibrosis follows.[33] Because cytoskeletal alterations of endothelial cells precede lymphocytic infiltration, a possibility may be that T-cell activation occurs in response to changes in the endothelial cells. It is also possible, however, that the changes in endothelial cell cytoskeleton occur in response to cytokines produced by T cells. Of note, IL-4 induces a collapse of vimentin intermediate filaments in cultured endothelial cells.[34] The central role of IL-4 in the pathophysiology of SSc is also supported by the observation that this cytokine induces human fibroblasts to synthesize elevated levels of extracellular matrix proteins, stimulates the growth of subconfluent fibroblasts, and promotes the chemotaxis of these cells.[35] The predominant activation of Th2-like T cells in SSc patients can also account for eosinophil infiltration in target organs, the increased levels of eosinophil cationic protein and eosinophil major basic protein,[36,37] as well as for polyclonal B-cell activation and autoantibody production, which is an early, nearly universal finding in these patients.[38]

The understanding of mechanisms determining the intense and persistent activation of Th2 cells, ie, the sustained

production of IL-4, in SSc is of considerable importance to our further knowledge of the etiopathogenesis of the disease. Both genetic and environmental factors are responsible for the Th1 or Th2 differentiation, although the mechanisms by which the genetic background controls the type of Th cell differentiation still remain elusive. There is general consensus that the presence of IL-4 at the time of antigen presentation is critical in determining the development of the naive Th cell into the Th2 pathway.[39] The source of IL-4, which is required for priming naive T cells to develop into Th2 cells, is still unclear. The major candidates include mast cells/basophils,[32,40] the $CD4^+NK1^+$ subset,[41] and the naive $CD4^+$ T cell themselves.[42] Thus, abnormal IL-4 production by one of these cell types may be responsible for the prevalent Th2 response in SSc patients. However, whether these alterations precede or they are secondary to, Th2-cell activation remains to be determined. An alternative but not mutually exclusive possibility is that SSc patients have a deficient activity of cytokines that inhibit the Th2-cell development, such as IFN-α/γ and IL-12.[43–46] In this respect, it is important to note that SSc patients exhibit reduced levels of IFN-γ in the blood as well as defective production of IFN-γ by both peripheral blood mononuclear cells and bronchoalveolar lavage cells.[7,47] Whatever mechanism is involved, the demonstration of a role for Th2-dominated responses in the pathophysiology of SSc may have potential therapeutic applications. Strategies aimed at inhibiting the development and/or function of Th2 cells may be envisaged.

References

1. Roumm AD, Whiteside TL, Medsger TA, Rodnan GP: Lymphocytes in the skin of patients with progressive systemic sclerosis. Arthritis Rheum 1984, 27:645–653
2. Prescott RJ, Freemont AJ, Jones CIP, Hoyland J, Fielding P: Sequential dermal microvascular and perivascular changes in the development of scleroderma. J Pathol 1992, 166:255–263
3. Fleischmajer R, Perlish JS, Reeves JRT: Cellular infiltrates in scleroderma skin. Arthritis Rheum 1977, 20:975–984
4. Lampert IA, Switters AJ, Janossy G, Thomas JA, Palmer S, Gordon-Smith E: Lymphoid infiltrates in skin in graft-versus-host disease. Lancet 1981, 2:1352–1358
5. Gonzalez-Amaro R, Alarcon-Segovia D, Alcocer Varela J, De Leon LD, Rosentstein Y: Mononuclear cell-fibroblast interactions in scleroderma. Clin Immunol Immunopathol 1988, 46:412–417
6. Famularo G, Procopio A, Giacomelli R, Danese C, Sacchetti S, Perego MA, Santoni A, Tonietti G: Soluble interleukin 2 receptor, interleukin 2 and interleukin 4 in sera and supernatants from patients with progressive systemic sclerosis. Clin Exp Immunol 1990, 81:368–372
7. Alms WJ, Wigley FM, Wise R: Immunological analysis of broncho alveolar lavage (BAL)cells from scleroderma patients and controls. Arthritis Rheum 1992, 35:S312
8. Needelman BW, Wigley FM, Stair RW: IL-1, IL-2, IL-4, IL-6 TNF-α, IFN-γ levels in sera from patients with scleroderma. Arthritis Rheum 1992, 35:67–72
9. Ihn H, Sato S, Fujimoto M, Kikuchi K, Takehara K: Demonstration of interleukin 2, interleukin 4 and interleukin 6 in sera from patients with localized scleroderma. Arch Dermatol Res 1995, 287:193–197
10. Mosmann TR, Coffman RL: TH1, and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989, 7:145–173
11. Romagnani S: Lymphokine production by human T cells in disease states. Annu Rev Immunol 1994, 12:227–257
12. Del Prete GF, De Carli M, Almerigogna F, Daniel KC, Zancuoghi MM, Vinante E, Pizzolo G, Romagnani S: Preferential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. FASEB J 1995, 9:81–86
13. Bengtsson A, Johansson C, Linder MT, Hallden G, van der Ploeg I, Scheynius A: Not only Th2 cells but also Th1 and Th0 cells express CD30 after activation. J Leukocyte Biol 1995, 58:683–689
14. Chilosi M, Facchetti F, Notarangelo LD, Romagnani S, Del Prete GF, Almerigogna F, De Carli M, Pizzolo G: CD30 cell expression, and abnormal soluble CD30 serum accumulation in Omenn's syndrome: evidence for a T helper 2-mediated condition. Eur J Immunol 1996, 26:329–334
15. Smith CA, Gruss H-J, Davis T, Anderson D, Farrah T, Baker E, Sutherland GR, Brannan CI, Copeland NG, Jenkins NA, Grabstein H, Gliniak B, McAllister IB, Fanslow W, Alderson M, Falk B, Gimpel S, Gillis S, Din WS, Goodwin R, Armitage RJ: CD30 antigen, a marker for Hodgkin's lymphoma: is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell 1993, 73:1349–1360
16. D'Elios MM, Romagnani P, Scaletti C, Annunziato F, Manghetti M, Mavilia C, Parronchi P, Pupilli C, Pizzolo G, Maggi E, Del Prete GF, Romagnani S: In vivo CD30 expression in human diseases with predominant activation of Th2-like T cells. J Leukocyte Biol 1997, 61:539–544
17. Del Prete GF, De Carli M, D'Elios MM, Maestrelli P, Ricci M, Fabbri L, Romagnani S: Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. Eur J Immunol 1993, 23:1445–1449
18. Maggi E, Mazzetti M, Ravina A, Annunziato F, De Carli M, Piccinni MP, Manetti R, Carbonari M, Pesce AM, Del Prete GF, Romagnani S: Ability of HIV to promote a Th1 to Th0 shift and to replicate preferentially in Th2 and Th0 cells. Science 1994, 265:244–248
19. Maggi E, Giudizi M-G, Biagiotti R, Annunziato F, Manetti R, Piccinni MP, Parronchi P, Sampognaro S, Giannarini L, Zuccati G, Romagnani S: Th2-like CD8+ cells showing B cell helper function, and reduced cytolytic activity in human immunodeficiency virus type 1 infection. J Exp Med 1994, 180:489–495
20. Pupilli C, Brunori M, Misciglia N, Selli C, Ianni L, Yanagisaw M, Mannelli M, Serio M: Presence and distribution of endothelin-1 gene expression in human kidney. Am J Physiol 1994, 267:F679–F687
21. Pupilli C, Ariel Gomez R, Tuttle JB, Peach MJ, Carey RM: Spatial association of renin-containing cells and nerve fibers in developing rat kidney. Pediatr Nephrol 1991, 5:690–695
22. Pizzolo G, Vinante F, Morosato L, Nadali G, Chilosi M, Gandini G, Sinicco A, Raiteri R, Semenzato G, Stein H, Pizzolo G: High serum level of soluble form of CD30 molecule in the early phase of HIV-1 infection as an independent predictor of progression to AIDS. AIDS 1994, 8:741–745
23. Del Prete GF, Maggi E, Pizzolo G, Romagnani S: CD30, Th2 cytokines, and HIV infection: a complex and fascinating link. Immunol Today 1995, 16:76–80
24. D'Elios MM, Manghetti M, De Carli M, Costa F, Baldari CT, Burroni D, Telford JL, Romagnani S, Del Prete GF: Th1 effector cells specific for Helicobacter pylori in the gastric antrum of patients with peptic ulcer disease. J Immunol 1997, 158:962–967
25. Parronchi P, Romagnani P, Annunziato F, Sampognaro S, Becchio A, Giannarini L, Maggi E, Pupilli C, Tonelli F, Romagnani S: Type 1 T helper (Th1)-cell predominance, and IL-12 expression in the gut of patients with Crohn's diseases. Am J Pathol 1997, 150:823–832
26. de Wit D, Van Mechelen M, Zanin C, Dautrelepont J-M, Velu T, Géerard C, Abramowicz D, Scheerlink JP, De Baetselier P, Urbain J, Leo O, Goldman M, Moser M: Preferential activation of Th2 cells in chronic graft-versus-host reaction. J Immunol 1993, 150:361–363
27. Finkelman FD: Relationships among antigen presentation, cytokines, immune deviation and autoimmune diseases. J Exp Med 1995, 182: 279–382
28. van der Heijden FL, Wierenga EA, Bos JD, Kapsenberg ML: High frequency of IL-4-producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. J Invest Dermatol 1991, 97:389–394
29. Nakamura T, Lee RK, Nam SY, Al-Ramadi BK, Koni PA, Bottomly K, Podack ER, Flavell RA: Reciprocal regulation of CD30 expression on CD4+ T cells by IL-4 and IFN-g. J Immunol 1997, 158:2090–2098
30. Annunziato F, Manetti R, Cosmi L, Galli G, Heusser CH, Romagnani S, Maggi E: Opposite role for interleukin-4 and interferon-γ on CD30 and lymphocyte activation gene-3 (LAG-3) expression by activated naive T cells. Eur J Immunol 1997, 27:2239–2244

31. Hamann D, Hilkens CMU, Grogan JL, Lens SMA, Kapsenberg ML, Yazdanbakhsh M, van Lier RAW: CD30 expression does not discriminate between human Th1-, and Th2-type T cells. J Immunol 1996, 156:1387–1391
32. Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews JP, Hunt TC, Okayama Y, Heusser CH, Bullock GR, Church MK, Holgate ST: Interleukin 4 is localized, and released by human mast cells. J Exp Med 1992, 176:1381–1386
33. White, B: Pathogenesis: immune aspects. Systemic Sclerosis. Edited by PJ Clements, ED Furst. Baltimore, Williams and Wilkins, 1996, pp 229–250
34. Klein NJ, Rigley KP, Callard RE: IL-4 regulated the morphology, cytoskeleton, and proliferation of human umbilical vein endothelial cells: relationship between vimentin and IL-4. Int Immunol 1992, 5:293–302
35. Postletwaite AE, Holness MA, Katai H, Raghow R: Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. J Clin Invest 1992, 90:1479–1485
36. Gustafsson R, Fredens K, Nettelbladt O, Hallgren R: Eosinophil activation in systemic sclerosis. Arthritis Rheum 1991, 34:412–422
37. Cox D, Earle L, Jimenez SA, Leiferman KM, Gleich GJ, Varga J: Elevated levels of eosinophil major basic protein in the sera of patients with systemic sclerosis. Arthritis Rheum 1995, 38:939–945
38. White B: Immunologic aspects of scleroderma. Curr Opin Rheumatol 1994, 6:612–615
39. Reiner SL, Seder RA: T helper cell differentiation in immune response. Curr Opin Immunol 1995, 7:360–366
40. Brunner T, Heusser CI, Dahinden CA: Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. J Exp Med 1993, 177:605–612
41. Yashimoto T, Paul WE: CD4pos, NK1.1pos T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3. J Exp Med 1994, 179:1285–1295
42. Croft M, Swain SL: Recently activated naive CD4 T cells can help resting B cells, and can produce sufficient autocrine IL-4 to drive differentiation to secretion of T helper 2-type cytokines. J Immunol 1995, 154:4269–4282
43. Hsieh C-S, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM: Development of TH1 $CD4^+$ T cells through IL-12 produced by Leisteria-induced macrophages. Science 1993, 260:547–549
44. Manetti R, Parronchi P, Giudizi MG, Piccinni M-P, Maggi E, Trinchieri G, Romagnani S: Natural killer cell stimulatory factor (interleukin-12) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. J Exp Med 1993, 177:1199–1204
45. Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinni M-P, Macchia D, Maggi E, Del Prete GF, Romagnani S: IL-4 and IFNs (α and γ) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. J Immunol 1992, 149:2977–2984
46. Wenner C, Guler ML, Macatonia SE, O'Garra A, Murphy KM: Roles of IFN-g and IFN-a in IL-12-induced Th1 development. J Immunol 1996, 156:1442–1447
47. Prior C, Haslam PL: In vivo, and in vitro production of interferon-γ in fibrosing interstitial lung diseases. Clin Exp Immunol 1992, 88:280–287