

## T Cell Interleukin-17 Induces Stromal Cells to Produce Proinflammatory and Hematopoietic Cytokines

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### Summary

Analysis of the cDNA encoding murine interleukin (IL) 17 (cytotoxic T lymphocyte associated antigen 8) predicted a secreted protein sharing 57% amino acid identity with the protein predicted from ORF13, an open reading frame of *Herpesvirus saimiri*. Here we report on the cloning of human IL-17 (hIL-17), the human counterpart of murine IL-17. hIL-17 is a glycoprotein of 155 amino acids secreted as an homodimer by activated memory CD4<sup>+</sup> T cells. Although devoid of direct effects on cells of hematopoietic origin, hIL-17 and the product of its viral counterpart, ORF13, stimulate epithelial, endothelial, and fibroblastic cells to secrete cytokines such as IL-6, IL-8, and granulocyte–colony-stimulating factor, as well as prostaglandin E<sub>2</sub>. Furthermore, when cultured in the presence of hIL-17, fibroblasts could sustain the proliferation of CD34<sup>+</sup> hematopoietic progenitors and their preferential maturation into neutrophils. These observations suggest that hIL-17 may constitute (a) an early initiator of the T cell–dependent inflammatory reaction; and (b) an element of the cytokine network that bridges the immune system to hematopoiesis.

**T** lymphocytes produce an array of small proteins that are involved in cell growth, inflammation, immunity, differentiation, and repair. These protein mediators referred to as cytokines are not produced constitutively by T cells, but rather are induced after receptor-mediated T cell activation (1, 2). Murine cytotoxic T lymphocyte associated antigen-8 (mCTLA8)<sup>1</sup>, a cDNA previously cloned by Rouvier et al. (3) from a T cell subtraction library, displays some of the features of a cytokine gene: in particular, a pre-

dicted hydrophobic NH<sub>2</sub>-terminal sequence that could correspond to a signal peptide (4) and the presence in its 3' untranslated region of AU-rich repeats associated with mRNA instability. Similar motifs have been found previously in mRNA of cytokines, growth factors, and oncogenes (5, 6). In addition to a predictable short half-life of its mRNA, the expression of mCTLA8 gene seemed to be tightly controlled since the corresponding transcripts could only be detected in the original T cell hybridoma after activation with PMA and ionomycin (PI) (3). Finally, mCTLA8 showed 57% homology with the predicted amino acid sequence of the open reading frame 13 (ORF13) of *Herpesvirus saimiri* (HVS) (3), a situation analogous to the presence of an IL-10 homolog in Epstein-Barr virus (7). We report here the cloning of the human counterpart of mCTLA8, human (h)IL-17, a cytokine produced only by activated memory T cells that induces stromal cells to secrete cytokines involved in inflammatory and hematopoietic processes.

<sup>1</sup>Abbreviations used in this paper: hIL-17, human IL-17; HVS, *Herpesvirus saimiri*; ORF13, open reading frame 13; mCTLA8, murine cytotoxic T lymphocyte-associated antigen 8; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PI, PMA and ionomycin.

Parts of this work were presented at the 1995 International Congress of Immunology on 24–31 July, San Francisco, CA, at the third Annual Conference of The International Cytokine Society on 12–15 September, 1995 in Harrogate, UK, and at the Cytokine and Cytokine Receptors AACR conference, on 14–18 October in Bolton Landing, NY. Portions of this work have appeared in abstract form (1995. *Cytokine*. 7:610).

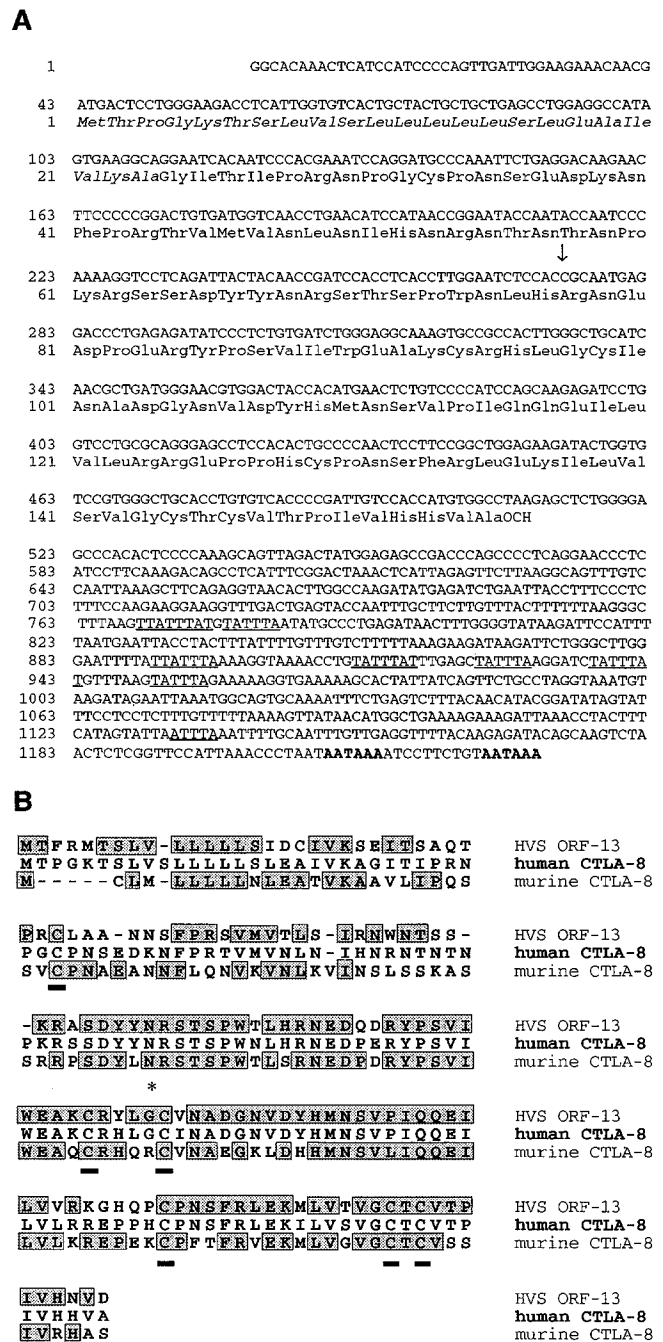
## Material and Methods

**Cells and Cell Lines.** PBMC were isolated from the blood of healthy donors, by Ficoll-Hypaque centrifugation. The CD4<sup>+</sup> cell-enriched population was prepared from PBMC by negative selection using a cocktail of mAbs against CD8, CD40, CD14, and NKH1 antigens and magnetic beads (Dynabeads, Dynal, Olso) coated with anti-mouse IgG. The CD8<sup>+</sup> cell-enriched population was prepared by the same method except that anti-CD8 mAb was replaced by anti-CD4 mAb. The B cell-enriched population was prepared by negative selection from normal tonsil using a cocktail of mAbs against CD8, CD4, CD14, and NKH1 antigens. The monocyte-enriched population was prepared from PBMC using plastic adherence. The purity of enriched populations was controlled by FACS<sup>®</sup> analysis (Becton Dickinson & Co., Mountain View, CA). PBMC or enriched populations were cultured in RPMI medium supplemented with 10% FCS and activated with a PI mixture of 5 ng/ml PMA and 3 µg/ml ionomycin, or 1 µg/ml Con A, or a combination of anti-CD28 and immobilized anti-CD3 mAbs (OKT3 plus anti-CD28). Concentrations of hIL-17 and IFN-γ secreted in the supernatants were determined by ELISA, and results represent the mean ± standard deviation of triplicate determinations.

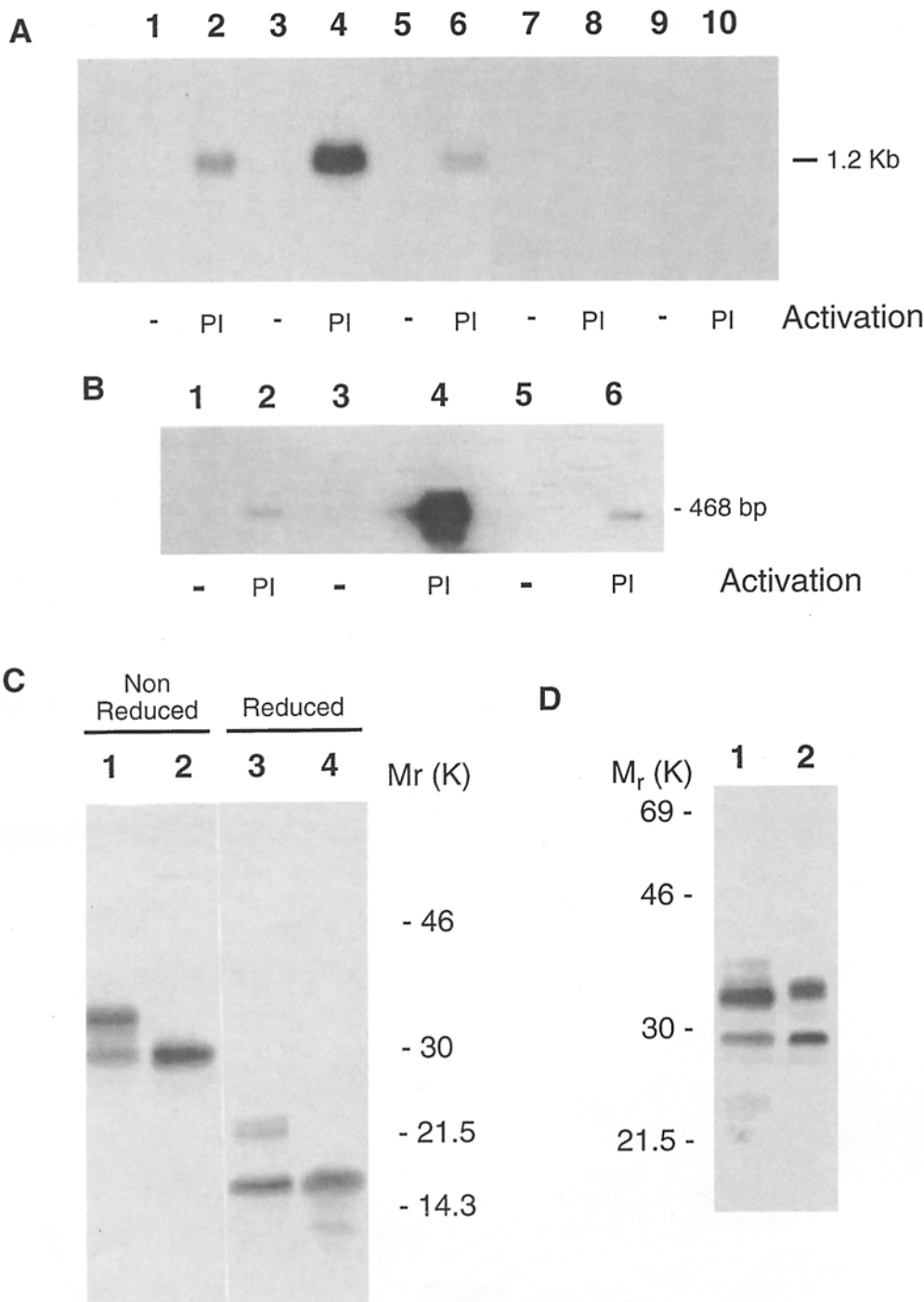
Rheumatoid synoviocytes were isolated from RA patients undergoing knee or wrist synovectomy and cultured as described before (8). Synovium pieces were finely minced and digested with 4 µg/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) in PBS Dulbecco's medium (GIBCO BRL, Gaithersburg, MD) for 2–3 h at 37°C. After centrifugation, cell suspension was resuspended in complete medium made of a-MEM (GIBCO BRL) with 2 mM L-glutamine, 100 U/ml penicillin, 50 mg/ml gentamicin, 20 mM Hepes buffer, and 2% Ultrosor (GIBCO BRL), and cultured in 150-cm<sup>2</sup> culture flasks (Falcon, Oxnard, CA). Nonadherent cells were removed and adherent cells were trypsinized at confluence and passaged. Synoviocytes used between passages 3 and 8 constituted a homogenous population of fibroblast-like cells, that were negative for CD2, CD19, CD14, CD45, and HLA-DR expression, as determined by FACSscan<sup>®</sup> analysis with FITC-conjugated mAbs from Becton Dickinson & Co.

CHA and TUMT epithelial cell lines of kidney carcinoma were kindly provided by C. Bain (Centre Léon Bérard, Lyon, France). MRC-5 human lung fibroblasts were obtained from the American Type Culture Collection (Rockville, MD). Bronchial epithelial cells were obtained from F. Gormond (Hôpital Lyon Sud, Lyon, France). These three cell lines were cultured in complete RPMI 1640 medium (GIBCO BRL), supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 50 mg/ml gentamicin, 20 mM Hepes buffer, and 10% heat-inactivated FCS. Human brain endothelial cells, kindly provided by Dr. G.E. Grau (Geneva University, Geneva, Switzerland), were cultured in MEM (GIBCO BRL), supplemented with 10% FCS, 2 mM glutamine, heparin (25 U/ml; Sigma Chemical Co., St. Louis, MO), and endothelial cell growth supplement (100 µg/ml, Sigma Chemical Co.). Human dermal fibroblasts, kindly provided by Dr. M.T. Zobot (Laboratoire de Biochimie, Hôpital Debrousse, Lyon, France), were cultured in complete Ham F-10 medium with 12% FCS.

**Generation of Mouse mAbs against hCTLA8.** Inbred BALB/c mice were immunized with three successive intraperitoneal injections of Freund's adjuvant (Sigma Chemical Co.) and 100 mg of purified hIL-17 obtained from supernatants of hIL-17-transfected NS0 cells. Spleens were removed for the fusion 3 d after a final i.v. injection of hIL-17. Hybridization was carried out using the nonsecreting myeloma cell line SP2/0-Ag8, with polyethylene glycol 1000 (Sigma Chemical Co.). Hybridoma supernatants were



**Figure 1.** Nucleotide sequence of the hIL-17 cDNA and comparison of its deduced polypeptide with mIL-17 and ORF13 of HVS. hIL-17 cDNA was cloned as described. (A) Nucleotide and deduced amino acid sequences of the hIL-17 cDNA. The first 23 amino acids constituting the signal peptide appear in italics. The exon sequenced from a genomic library starts at nucleotide number 274 (↓), and the eight ATTTA repeats are underlined. The genomic sequence contains two polyadenylation signals that are in bold characters. (B) Sequence alignment of proteins encoded by human and murine IL-17 cDNAs and ORF13 of HVS. Murine human and viral (40) protein sequences were aligned using the Meg Align program (DNASTAR Inc., Madison, WI). Regions of protein identity with the human sequence are boxed, and the six conserved cysteine residues are underlined. The N-glycosylation site (Asn-X-Ser) conserved between the three proteins is indicated by an asterisk. The cDNA sequence data are available from EMBL/GenBank/DBJ under accession number Z58820.



**Figure 2.** (A) hIL-17 mRNA transcripts are detected in activated but not resting CD4<sup>+</sup> T cells. PBMC (lanes 1 and 2), purified CD4<sup>+</sup> T cells (71% CD4<sup>+</sup>, 8% CD8<sup>+</sup>, 6% CD14<sup>+</sup>, 7% CD20<sup>+</sup>; lanes 3 and 4), purified CD8<sup>+</sup> T cells (70% CD8<sup>+</sup>, 3% CD4<sup>+</sup>, 2% CD14<sup>+</sup>, 11% CD20<sup>+</sup>; lanes 5 and 6), purified B cells (93% CD19<sup>+</sup>, 6% CD4<sup>+</sup>, 1% CD8<sup>+</sup>, 0% CD14<sup>+</sup>; lanes 7 and 8) and purified monocytes (73% CD14<sup>+</sup>, 15% CD20<sup>+</sup>, 0% CD4<sup>+</sup>, 0% CD8<sup>+</sup>; lanes 9 and 10) were activated (PI) or not (-) for 4 h with a mixture of 5 ng ml<sup>-1</sup> PMA and 3 μg ml<sup>-1</sup> ionomycin. Total RNAs were analyzed on Northern blot with a <sup>32</sup>P-labeled hIL-17 probe. (B) Upon activation with PI, expression of hIL-17 mRNA was restricted to the CD4<sup>+</sup>CD45RO memory T cells subset. Blots of hIL-17 RT-PCR products obtained after 25 cycles of amplification from PI-activated (PI) and nonactivated (-) peripheral CD8<sup>+</sup> T cell-enriched population (lanes 1 and 2), tonsillar CD4<sup>+</sup>CD45RO<sup>+</sup> (92% CD4<sup>+</sup>, 2% CD8<sup>+</sup>, 2% CD19<sup>+</sup>, 2% CD14<sup>+</sup>; lanes 3 and 4) and CD4<sup>+</sup>CD45RA<sup>+</sup> T cells (90% CD4<sup>+</sup>, 2% CD8<sup>+</sup>, 2% CD19<sup>+</sup>, 2% CD14<sup>+</sup>; lanes 5 and 6) were hybridized with the hIL-17 probe and autoradiographed for 5 h. The expected size of the PCR product was 468 bp. (C) Human hIL-17 is a N-glycosylated dimer. Purified <sup>125</sup>I-labeled hIL-17 was digested with endoglycosidase-F (lanes 2 and 4) or not (lanes 1 and 3) before migration on reduced and nonreduced SDS-PAGE. (D) Metabolically labeled natural hIL-17 secreted by activated CD4<sup>+</sup> T cells (lane 1) and recombinant hIL-17 produced by transfected NS0 cells (lane 2) appear similar upon SDS-PAGE.

harvested after 6 d, incubated on hIL-17-transfected COS-7 cells coated in 96-well plates for 30 min at 37°C. Antibody binding was then revealed with peroxidase-conjugated sheep anti-mouse IgG (Biosys, Compiègne, France) at a 1:200 dilution in 2% human serum/PBS for 1 h at room temperature. Positive hybridomas were cloned by a limiting dilution method, expanded, and produced in ascites from pristane-treated BALB/c mice. After sodium sulphate precipitation of ascites, the mAbs were purified by anion-exchange chromatography on a Zephyr-D silicium column (IBF Sepracor, Villeneuve, France).

**Cloning of hIL-17.** A human genomic library (Clontech, Palo Alto, CA) was screened using the mCTLA8 cDNA as described previously (3). For the cDNA library, PBMC (10<sup>6</sup>/ml) were stimulated for 4 h with PI before extraction of total RNAs. cDNA synthesis and cloning was performed with a Superscript Plasmid System (GIBCO-BRL) and screened with a probe made of the coding region of hIL-17-terminal exon.

**Northern Blot and Reverse Transcription-PCR Analyses.** CD4<sup>+</sup>, CD8<sup>+</sup>, B cells, and monocyte-enriched populations were activated for 4 h with PI before total RNAs were extracted by the

single-step method (9). Northern blots were performed with 10  $\mu$ g of total RNA/sample as described (10). hIL-17 transcripts were detected with a  $^{32}$ P-labeled probe corresponding to the hIL-17 3' exon. CD4<sup>+</sup>CD45RO and CD4<sup>+</sup>CD45RA cells were purified from tonsil CD4<sup>+</sup> cell-enriched populations by negative selection using anti-CD45RO or anti-CD45RA antibodies, respectively. For reverse transcriptase (RT)-PCR, total RNAs were reverse transcribed using an oligo-dT primer; half of each cDNA sample was PCR amplified (25 cycles of 1 min at 95°C, 2 min at 50°C, and 3 min at 72°C) using two 24-nucleotide primers that hybridize to both ends of the hIL-17 coding region. To ensure equal amounts of total RNA between samples, the second half of the cDNA samples was PCR amplified under the same conditions using human  $\beta$ -actin-specific primers (Stratagene Inc., La Jolla, CA), yielding a product of 661 bp visualized by ethidium bromide staining (not shown). The expected size of the hIL-17 PCR product is 468 bp.

**Production and Purification of hIL-17- and ORF13-encoded Proteins.** Large amounts of recombinant hIL-17- and ORF13-encoded proteins were obtained from the supernatants of NS0 cells stably transfected with the expression plasmid pEE12 containing the appropriate coding sequences inserted between the EcoR1 and Bcl1 restriction sites. The secreted hIL-17 was purified from the supernatant by a three-step chromatographic process. The first step took advantage of the presence of seven His

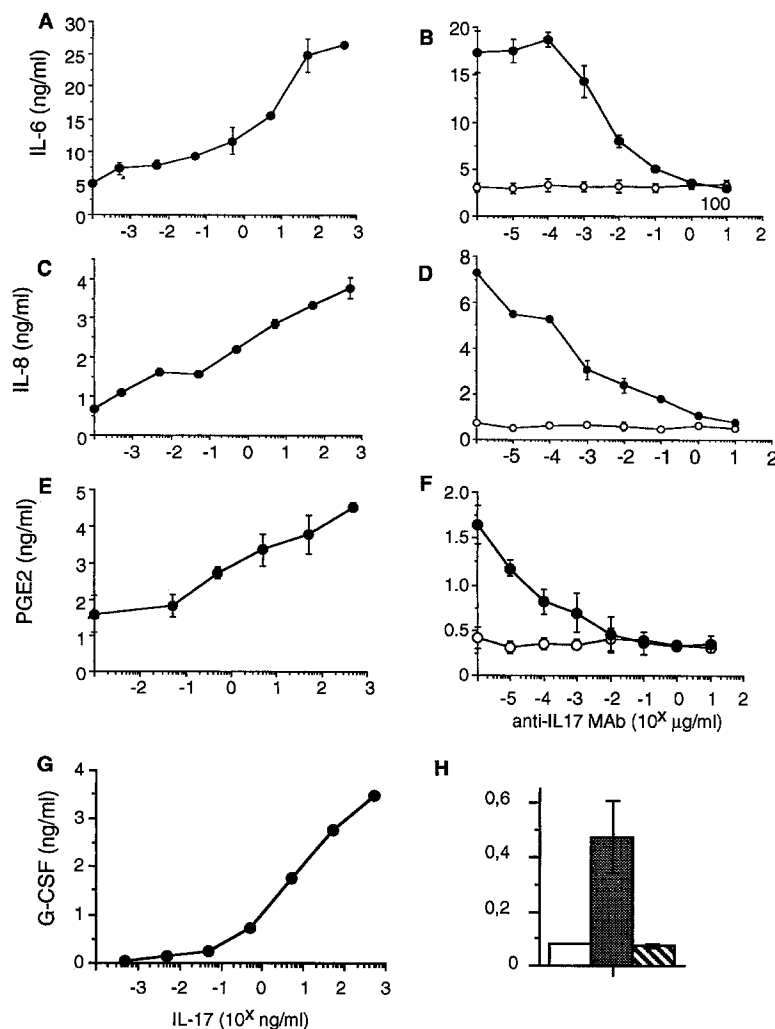
**Table 1.** Secretion of hIL-7 by Human PBMC after Activation

Cell type	Activation	Time	hIL-17	IFN- $\gamma$
		h	ng/ml	
PBMC*	None	72	<1.6	ND
	PI	16	6.8 $\pm$ 0.9	15.4 $\pm$ 0.9
		48	20 $\pm$ 2.7	16.6 $\pm$ 1.4
		72	22.7 $\pm$ 1.4	16.5 $\pm$ 1.7
	Con A	72	6.9 $\pm$ 0.2	ND
OKT3 + anti-CD28	72	3.8 $\pm$ 0.3	ND	
CD4 <sup>+</sup>	PI	72	8.9 $\pm$ 0.8	ND
CD8 <sup>+</sup>	PI	72	<1.6	ND

\*Donor 1.

†Donor 2.

residues to use a Zn ion affinity column (11). Elution from this column was performed with a gradient of 20–100 mM Imidazole buffer. Fractions containing hIL-17 were then further purified by anion exchange HPLC followed by a third step of gel filtration



**Figure 3.** Biological activities of hIL-17. Recombinant hIL-17 increased the secretion of IL-6 (A), IL-8 (C), PGE<sub>2</sub> (E), and G-CSF (G) secretions by rheumatoid synovial fibroblasts; this effect was completely suppressed in the presence of a hIL-17-specific mAb (B, D, F, and H). Cells were cultured for 48 h in various concentrations of purified anti-hIL-17 mAb5 without hIL-17 (open circles), or in the presence of 50 ng/ml hIL-17 (closed circles, B, D, F). In H, cells were cultured for 48 h in culture medium alone (white column), in 50 ng/ml of purified hIL-17 (dark column), or in 50 ng/ml of purified hIL-17 preincubated for 30 min at 37°C with 1  $\mu$ g/ml of anti-hIL-17 mAb5 (hatched column). Results represent the mean  $\pm$  SD of triplicate determinations from one representative experiment.

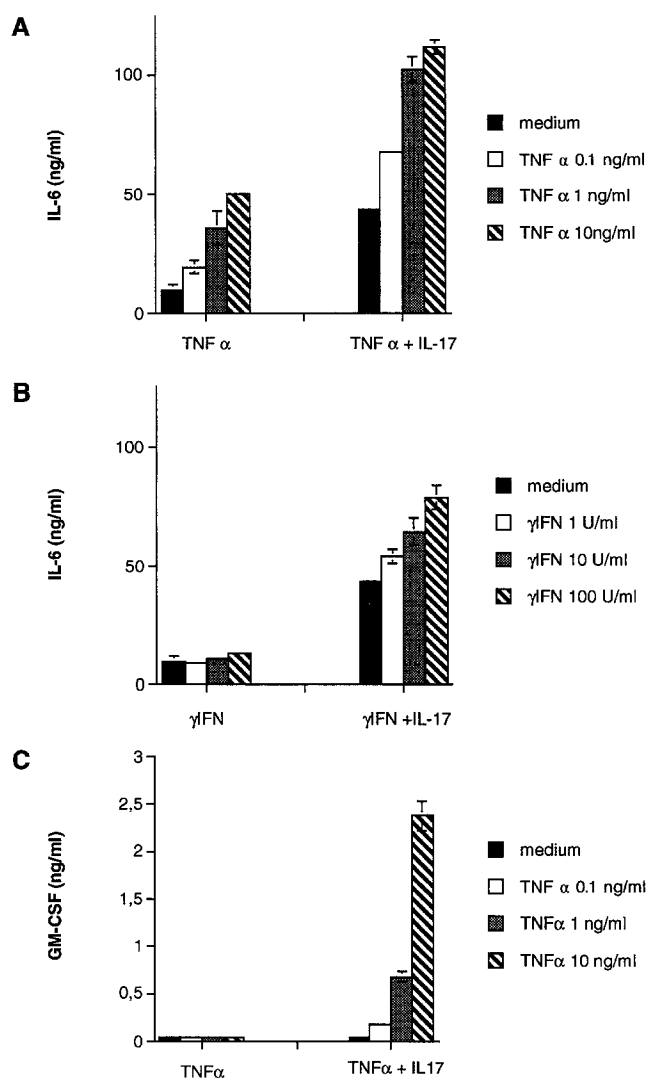
onto a Superdex G-75 column (Pharmacia, Uppsala, Sweden). The absence of bacterial endotoxin was verified using the Limulus Amebocyte Lysate assay (BioWhittaker, Walkersville, MD). ORF13-encoded protein was purified by affinity chromatography using the anti-IL-17 mAb5 immobilized to Affigel 10 (Bio-Rad Laboratories, Hercules, CA). Purified hIL-17 was labeled with  $^{125}\text{I}$  as previously described (12, 13) and nontreated or endoglycosidase-F (Boehringer Mannheim, Mannheim, Germany)-digested material was submitted to SDS-PAGE under reducing and nonreducing conditions. A CD4<sup>+</sup> cell-enriched cell population was activated with PI for 4 h and then labeled overnight with [ $^{35}\text{S}$ ]methionine and [ $^{35}\text{S}$ ]cysteine. Transfected NS0 cells were radiolabeled following the same protocol. Secreted proteins were immunoprecipitated with anti-hIL-17 mAb and protein G-Sepharose (Sigma Chemical Co.) before comparison by SDS-PAGE.

**Biological Assays.** Synoviocytes, fibroblasts, and epithelial or endothelial cell lines ( $10^4$  cells/well) were incubated in 96-well plates (Falcon) in a final volume of 250  $\mu\text{l}$  of their respective complete culture medium. Each cytokine or hIL-17 at indicated concentrations was added at the onset of the culture. Unless otherwise indicated, cell-free supernatants were collected after 48 h, and stored at  $-20^\circ\text{C}$  until cytokine assays. Concentrations of IL-6, IL-8, G-CSF, GM-CSF, and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) were measured by commercial two-site sandwich ELISA (Medgenix Diagnostics, Fleurus, Belgium or R&D Systems Inc., Minneapolis, MN). PGE<sub>2</sub> levels were measured using a commercial radioimmunoassay (DuPont NEN, Boston, MA). Biological activities were blocked by preincubation at  $37^\circ\text{C}$  for 30 min with 1  $\mu\text{g}/\text{ml}$  of the anti-hIL-17 mAb5.

**Cocultures of Human Hematopoietic Progenitors with Irradiated Synoviocytes.** Human hematopoietic progenitor cells were isolated from cord blood by positive selection using anti-CD34 mAb coupled to magnetic beads (Miltenyi Biotec, Sunnyvale, CA) as described before (14). Typically, the purity of CD34<sup>+</sup> cells ranged from 60 to 90%. Rheumatoid synovial fibroblasts were seeded at a density of  $10^4$  cells/well in a 96-well plate and irradiated at 5,000 rad the day after. Purified hIL-17 or the protein control was then added at the same time as the CD34<sup>+</sup> cells ( $10^4$  cells/well). Protein control was a supernatant of mock-transfected NS0 cells purified by the same procedure as hIL-17. The culture medium including hIL-17 or the protein control was renewed every fifth day. After 8, 12, and 15 d, nonadherent cells were resuspended from four different wells of each coculture condition and viable cells (as judged by trypan blue exclusion) were enumerated in a hemacytometer (Reichert, Buffalo, NY). Results represent the mean  $\pm$  standard deviation of duplicate determinations from four independent experiments performed with synovial fibroblasts and CD34<sup>+</sup> cells from different donors. Since the CD34<sup>+</sup>-enriched population also contains nonnegligible amounts of CD34<sup>-</sup> cells, the phenotype of proliferating cells was investigated. For this purpose, after 3 d of coculture, nonadherent cells were double-labeled with FITC-conjugated anti-CD34 antibody (Immunotech, Marseille, France) and Hoechst reagent 33342 (0.5  $\mu\text{g}/\text{ml}$ ; Calbiochem-Novabiochem Corp., La Jolla, CA) and analyzed by FACS<sup>®</sup>.

## Results and Discussion

**Cloning of hIL-17.** The screening of a human genomic library with mCTLA8 cDNA yielded one clone that contained the 3'-terminal exon and untranslated region of hIL-17 (Fig. 1 A). Messenger RNA analysis with this human probe demonstrated the expression of a single 1.2-kb hIL-17



**Figure 4.** Cooperation between hIL-17 and TNF- $\alpha$  or IFN- $\gamma$  for the secretion of IL-6 and GM-CSF by rheumatoid synoviocytes. These cells ( $10^4$  cells/well) were incubated with serial dilutions of IFN- $\gamma$  (1–100 U/ml) or TNF- $\alpha$  (0.1–10 ng/ml) in presence or absence of hIL-17 (5 ng/ml). After 48 h, the levels of IL-6 (A and B) and GM-CSF (C) in the supernatants were measured by ELISA. Results represent the mean  $\pm$  SD of triplicate determinations from one representative experiment.

transcript in PBMC activated with PI (Fig. 2 A). Using the same probe, three full-length clones encoding hIL-17 were isolated from a cDNA library prepared from activated PBMC (Fig. 1 A). The ORF codes for a polypeptide of 155 amino acids that contains a hydrophobic NH<sub>2</sub> terminus, corresponding most likely to the signal peptide of secreted proteins (4), and a single N-glycosylation site at Asn68. Like the 3' untranslated region of mCTLA8 and of several cytokines, the human sequence presents eight copies of the ATTTA motif involved in the rapid decay of mRNA (5, 6). The hIL-17 protein displays more homology to HVS ORF13 (66%) than to mCTLA8 (58%), whereas the three sequences show conservation of the six cysteine residues

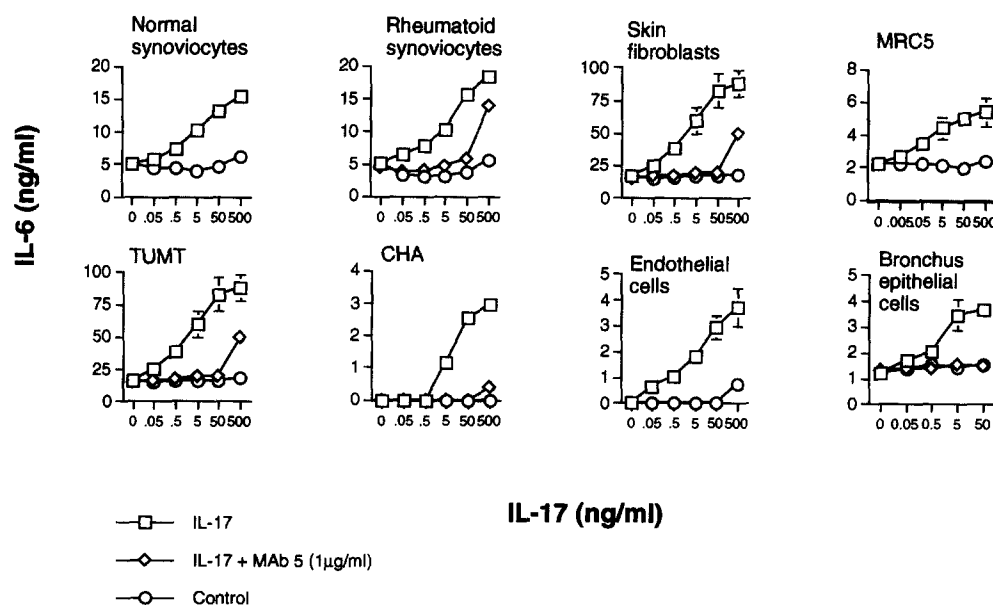
and of the N-glycosylation site (Fig. 1 B). Therefore, the amino acid sequence of IL-17 is well conserved between species.

**hIL-17 mRNA Is Expressed Mainly in Activated CD4<sup>+</sup> Memory T Cells.** Northern blot analysis could not detect hIL-17 transcripts in resting PBMC, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, B cells, monocytes (Fig. 2 A), or in various fetal (heart, brain, lung, liver, and kidney) or adult (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and PBL) human organs (not shown). After a 4-h activation with PI, hIL-17 transcripts were found mainly in CD4<sup>+</sup>-activated T cells (Fig. 2 A). The faint signal observed in activated CD8<sup>+</sup> T cells may be due to residual CD4<sup>+</sup> T cells. Activated B cells and monocytes did not express hIL-17 mRNA. Among activated CD4<sup>+</sup> T cells, the CD45RO<sup>+</sup> subset was the major source of hIL-17 mRNA as demonstrated after 25 cycles of PCR amplification (Fig. 2 B). hIL-17 transcripts could be detected in freshly isolated tonsillar T cells after 35 cycles of PCR amplification (not shown). Thus, hIL-17 transcripts can be detected only in T lymphocytes upon activation, and then mostly, if not only, in activated CD4<sup>+</sup>CD45RO<sup>+</sup> memory T cells.

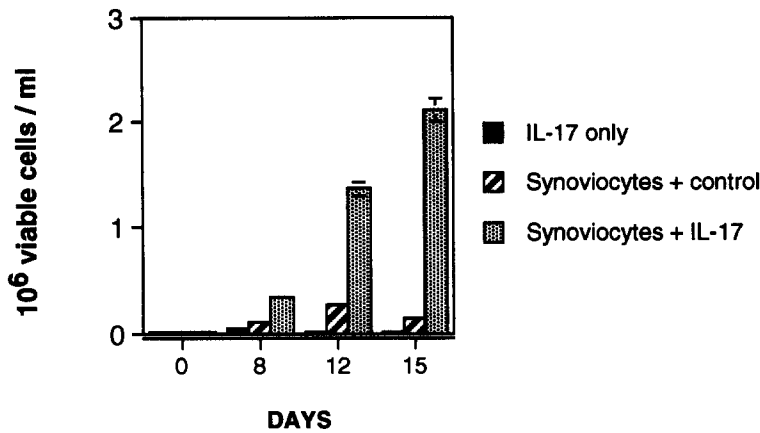
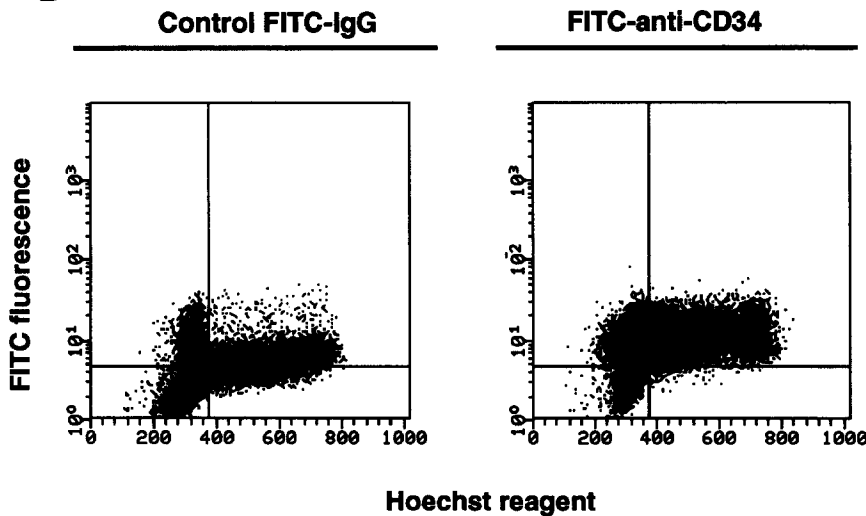
**hIL-17 Is Secreted by Activated CD4<sup>+</sup> T Cells as a Mixture of Homodimeric Glycosylated and Nonglycosylated Polypeptides.** Culture of mouse NS0 cells transfected with the plasmid expression vector PEE12 containing the hIL-17 full-length cDNA in the presence of [<sup>35</sup>S]methionine and [<sup>35</sup>S]cysteine resulted in the specific secretion of two labeled proteins with apparent molecular weights of 28,000 and 31,000 on SDS-PAGE. Under reducing conditions, recombinant hIL-17 migrated as two compounds of 15 and 22 kD (Fig. 2 C). Digestion of purified recombinant hIL-17 with endoglycosidase F yielded the 28- (nonreducing) and 15-kD (reducing) species, suggesting that the higher molecular weight species represent N-glycosylated forms (Fig. 2 C).

NH<sub>2</sub>-terminal amino acid sequencing of the purified 28- and 31-kD bands revealed an identical sequence starting at Gly24. Thus, recombinant hIL-17 is secreted, after cleavage of a 23-amino acid signal peptide, as a mixture of glycosylated and unglycosylated covalently bound homodimers. Immunoprecipitation with hIL-17-specific mAbs of <sup>35</sup>S-labeled supernatants shows that activated CD4<sup>+</sup> T cells and hIL-17 transfected NS0 cells both secreted a mixture of glycosylated and unglycosylated hIL-17 (Fig. 2 D). hIL-17 was detected by ELISA in the supernatant of PI-activated PBMC after 16 h of culture, and its concentration reached a plateau after 48 h (Table 1). Consistent with the strong hIL-17 mRNA signal, supernatants of PI-activated CD4<sup>+</sup> T cells or PBMC were found to contain  $\leq 22.7$  ng/ml hIL-17, indicating that this cytokine was a major product of these activated T cells (Table 1). Con A or OKT3 plus anti-CD28 mAb activations both induced PBMC to secrete hIL-17, although less than PI activation (Table 1). When purified CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets were activated with PI, hIL-17 was detected in supernatants of activated CD4<sup>+</sup> T cells exclusively (Table 1), thus confirming at the protein level that hIL-17 is secreted by activated CD4<sup>+</sup> T cells.

**hIL-17 Induces Stromal Cells to Secrete Inflammatory and Hematopoietic Cytokines.** The properties of purified recombinant hIL-17 were assayed on various immune and non-immune cell populations. hIL-17 had no major effect on the proliferation, cytokine secretion (IFN- $\gamma$ , IL-4, IL-6, IL-10), phenotype (CD3, CD4, CD8), or cytotoxicity of total PBMC or purified CD4<sup>+</sup> and CD8<sup>+</sup> T cells, regardless of whether these cells had been activated with PHA, tetanus toxoid, or IL-2. hIL-17 did not affect the proliferation and Ig production of normal tonsillar B cells activated either through antigen receptor or through CD40. hIL-17 did not induce proliferation or differentiation of cord blood CD34<sup>+</sup> hematopoietic progenitors cultured with or with-



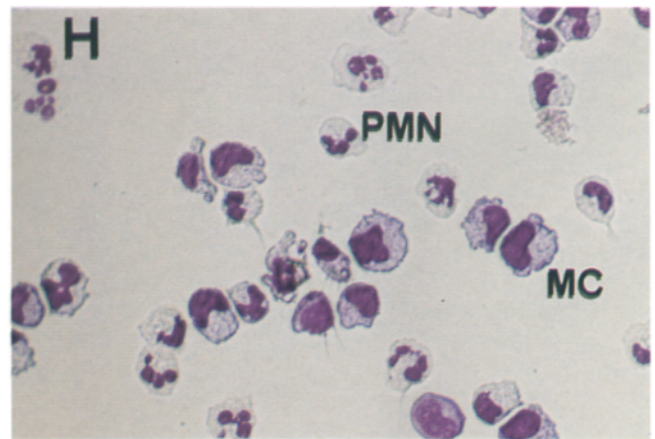
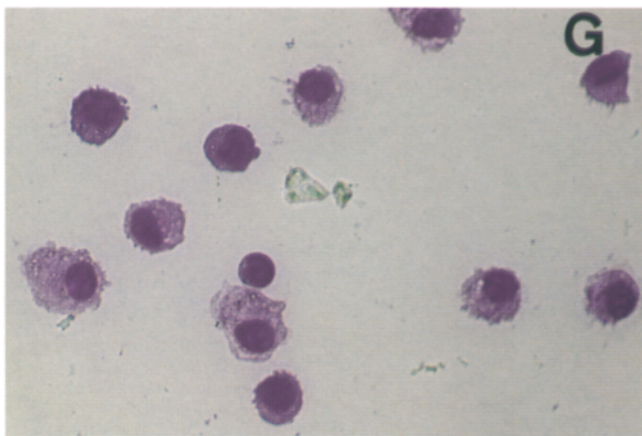
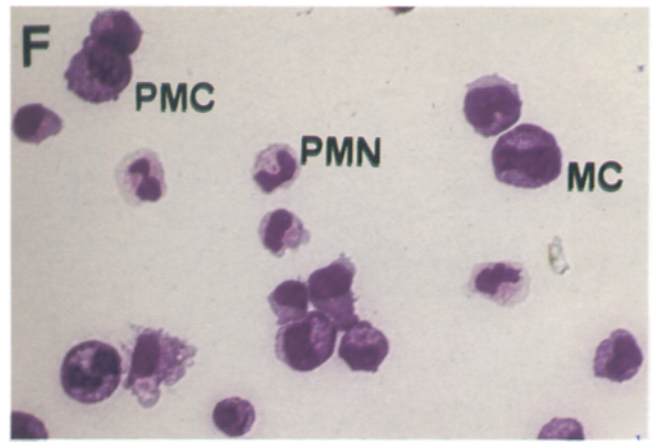
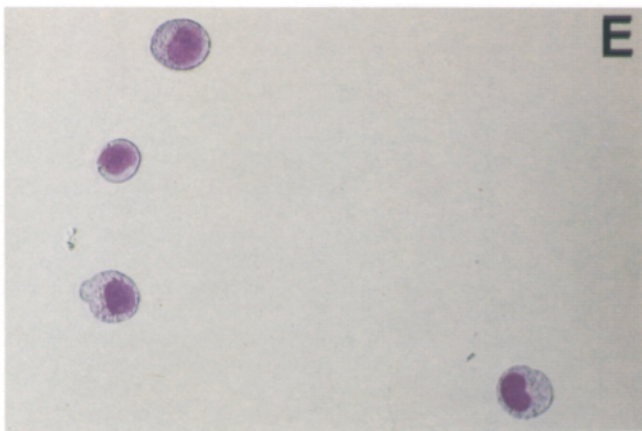
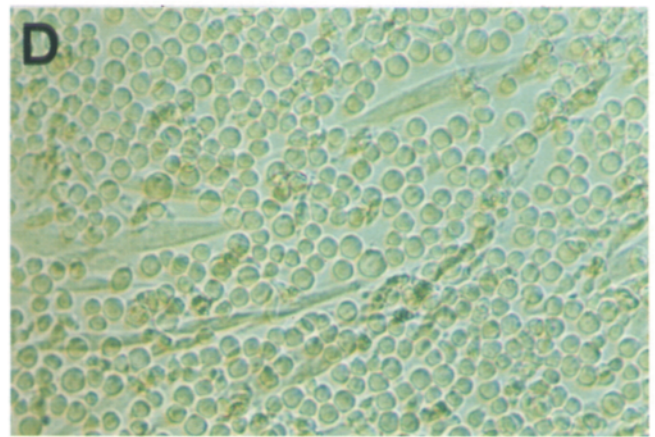
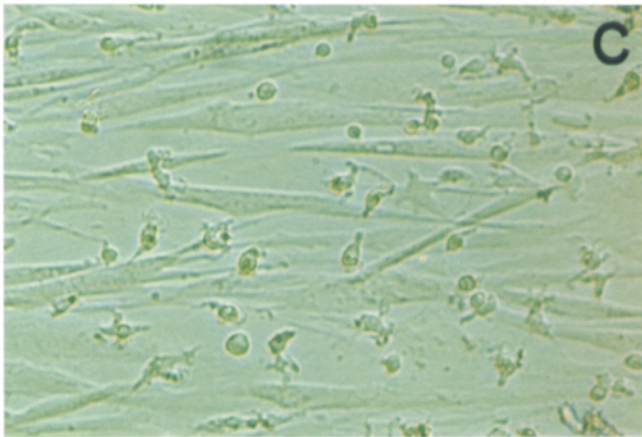
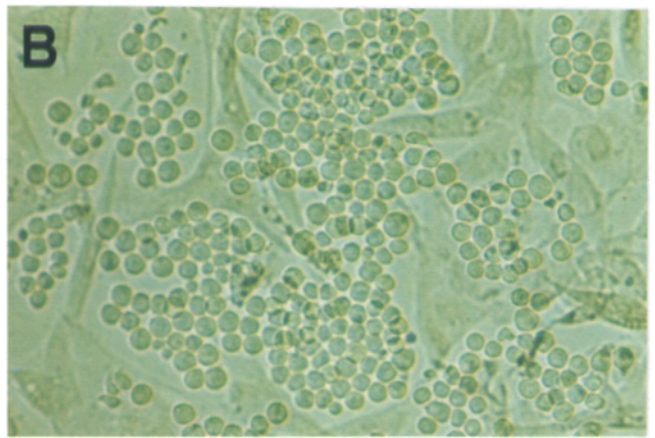
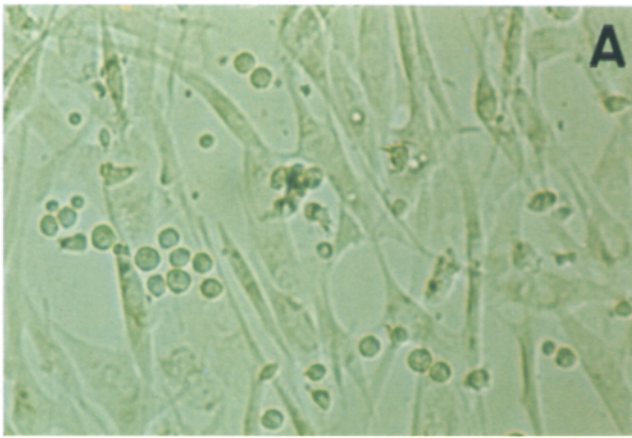
**Figure 5.** Many types of cells respond to hIL-17 by an increased secretion of IL-6. Cells ( $10^4$ /ml) were incubated for 48 h with 0–500 ng/ml of hIL-17 in absence (squares) or presence (diamonds) of 1 μg/ml of mAb5. Corresponding dilutions of a supernatant from mock-transfected NS0 cells purified by the same process as hIL-17 was used as a control (circles).

**A****B**

**Figure 6.** (A) Effect of hIL-17 (100 ng/ml, dark and light gray columns) or protein control (hatched columns) on the growth of CD34<sup>+</sup> cells cocultured with (hatched and light gray columns) or without (dark grey columns) 10<sup>5</sup>/ml irradiated synoviocytes (5,000 rads). (B) FACS<sup>®</sup> analysis of non adherent cells harvested after 3 d of coculture of purified CD34<sup>+</sup> human progenitor cells with irradiated synoviocytes in the presence of IL-17. (Right) Plot corresponds to cells double-labeled with Hoechst reagent 33342 and FITC-conjugated anti-CD34; (left) plot corresponds to a double labeling with Hoechst reagent 33342 and FITC-conjugated control antibody.

out GM-CSF (not shown). In contrast, hIL-17 induced the secretion of IL-6, IL-8, PGE<sub>2</sub>, and G-CSF from primary cultures of synovial fibroblasts (Fig. 3, A, C, E, and G). The effect of hIL-17 on cytokine and PGE<sub>2</sub> production by fibroblasts was dose dependent with a half-maximal effect at 3 ng/ml, and a maximal effect at 20 ng/ml, suggesting high affinity interaction with surface receptors (Fig. 3, A, C, E, and G). These effects were specific as they were blocked by an anti-hIL-17 mAb (Fig. 3, B, D, F, and H). The same mAb did not interfere with the IL-1β- as well as LPS-induced secretion of IL-6 by synoviocytes, thus dem-

onstrating its binding specificity (not shown). Purified ORF13 displays the IL-6- and IL-8-inducing activities of hIL-17 and could also be blocked by the anti-hIL-17 neutralizing mAb (not shown). Thus, hIL-17 (as well as its viral counterpart) turned out to be a cytokine-inducing cytokine. When various combinations of cytokines were tested, both TNF-α and IFN-γ were found to have an additive effect on the hIL-17-induced secretion of IL-6 (Fig. 4, A-B). Moreover, whereas neither hIL-17 nor TNF-α alone had any effect on the secretion of GM-CSF, the combination of these two cytokines induced synovial fibroblasts to pro-





duce GM-CSF (Fig. 4 C). Normal synoviocytes and other adherent cells from various human tissues were tested for their capacity to respond to hIL-17. Kidney carcinoma epithelial cell lines (CHA and TUMT), primary cultures of skin fibroblasts and brain endothelial cells, embryonic lung fibroblasts (MRC5), and a bronchial epithelial cell line transformed by SV40, showed an increased secretion of IL-6 specifically induced by hIL-17 (Fig. 5). Interestingly, hIL-17 did not alter the secretion of these cytokines by blood monocytes (not shown). These observations suggest that stromal cells from most tissues would respond similarly to the IL-17 produced by activated T cells.

*Human hIL-17 Induces Fibroblasts to Support the Growth and Differentiation of CD34<sup>+</sup> Hematopoietic Progenitors.* As G-CSF (15–17) and IL-6 (18, 19) are important hematopoietic growth factors, hIL-17 was tested for its effects in coculture of CD34<sup>+</sup> hematopoietic progenitor cells and synoviocytes. The latter are known to support the growth of CD34<sup>+</sup> progenitor cells as efficiently as primary bone marrow stromal cell lines (20). As shown in Fig. 6 A, CD34<sup>+</sup> progenitor cells proliferated moderately when cultured with irradiated human fibroblasts. Addition of hIL-17 resulted in a strong enhancement of this proliferation, with a 6–10-fold increase in cell number after 15 d of culture. FACS<sup>®</sup> analysis after 3 d of coculture showed that the only cells to enter the cell cycle were CD34<sup>+</sup> (Fig. 6 B). Microscopic examination of cultures demonstrated the presence of foci of proliferating round cells after 8 d (Fig. 7 B) and a homogenous layer of round cells after 12 d (Fig. 7 D). May-Grünwald Giemsa staining showed that over 50% of cells generated during 12 d in the presence of hIL-17 had the morphological characteristics of mature neutrophils (Fig. 7, F and H).

## Conclusion

This report describes the molecular cloning, expression pattern, biochemical characterization, and biological activities of hIL-17, a cytokine specifically expressed by activated memory T cells that turns on the secretion of proinflammatory and hematopoietic cytokines by stromal cells. After hIL-10, hIL-17 is another example of an interleukin that has been hijacked by a virus probably as a way to escape the host-immune response. Interestingly, as both the IL-8R gene cluster and the hIL-17 gene map on human chromosome 2q31-q35 (3, 21), the present finding of hIL-17 inducing IL-8 and the capture of these two genes by HVS

suggest the possibility of a functional relationship between IL-8R and hIL-17.

When compared with IL-2 and IFN- $\gamma$ , which are specifically produced by both CD4<sup>+</sup> and CD8<sup>+</sup> T cells, the cellular source of IL-17 appears to be even more restricted, inasmuch as only activated CD4<sup>+</sup> memory T cells express it. Yet, supernatant of activated blood T cells contains large amounts of this cytokine ( $\leq 20$   $\mu\text{g/ml}$ ). It will be important to determine whether this secreting population displays a specific phenotype (e.g., Th1 vs. Th2) and plays a specific role in some pathological conditions.

In conclusion, the induction of secretion by stromal cells of IL-6, IL-8, and PGE<sub>2</sub> but not of IL-1 or TNF, and the lack of detectable activity on monocytes suggest a limited proinflammatory role of IL-17 in T cell-driven inflammatory pathological processes such as psoriasis or sarcoidosis, that is currently being analyzed. Moreover, activated CD4<sup>+</sup> T cells produce hIL-17, which stimulates stromal cells to produce a range of cytokines including IL-6 and G-CSF, leading to hematopoiesis of neutrophils in particular. The unique cellular source (activated T cells) of hIL-17 and its capacity to induce the secretion by stromal cells of IL-6, IL-8, and G-CSF seem tailored to contribute to the inducible hematopoiesis observed after antigenic stimulation (1, 22). Thus, activated T cells may affect hematopoietic progenitors both directly through the production of IL-3 (23–25), IL-4 (26–28), IL-5 (29–31), and GM-CSF (32, 33), as well as indirectly through the secretion of hIL-17. Not only could hIL-17 have a long-term effect on hematopoiesis by inducing G-CSF production, but, through the induction of IL-6 and IL-8 release by stromal cells, it may also participate in the triggering of the acute neutrophilia (18, 34) that permits a prompt nonspecific immune response against infectious agents (25, 35, 36). Incidentally, the indirect hematopoietic activity of hIL-17 might partially explain why removal of T cells from donor marrow leads to a significant reduction of engraftment (37, 38). Whether IL-17 represents a redundancy of the immune system or plays a crucial role in the inflammatory response and hematopoiesis will be addressed by the knocking out of this gene.

While this work was being submitted for publication, another group also reported the cloning of hIL-17, the two sequences being 100% identical (39). The availability of highly purified recombinant hIL-17 will permit further characterization of the full spectrum of their biological activities.

**Figure 7.** Effect of hIL-17 on the growth and maturation of CD34<sup>+</sup> cells cocultured with irradiated synovial fibroblasts. Cocultures incubated for 8 d (A, B, E, and F) or 12 d (C, D, G, and H) with hIL-17 (B, D, F, and H) or with the protein control (A, C, E, and G) were observed under phase contrast microscope ( $\times 200$ ) (A–D) and after May-Grünwald Giemsa staining. Cells were identified as promyelocyte (PMC), myelocyte (MC), and polymorphonuclear neutrophil (PMN).

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