The Journal of Immunology

IL-17 Stimulates Intraperitoneal Neutrophil Infiltration Through the Release of GRO α Chemokine from Mesothelial Cells

Janusz Witowski, Krzysztof Pawlaczyk, Andrzej Breborowicz, Axel Scheuren, Malgorzata Kuzlan-Pawlaczyk, Justyna Wisniewska, Alicja Polubinska, Helmut Friess, Gerhard M. Gahl, Ulrich Frei and Achim Jörres

J Immunol 2000; 165:5814-5821; ;
doi: 10.4049/jimmunol.165.10.5814
http://www.jimmunol.org/content/165/10/5814

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

IL-17 Stimulates Intraperitoneal Neutrophil Infiltration Through the Release of GROα Chemokine from Mesothelial Cells[1]

Janusz Witowski,*† Krzysztof Pawlaczyk,* Andrzej Breborowicz,* Axel Scheuren,† Malgorzata Kuzlan-Pawlaczyk,* Justyna Wisniewska,* Alicja Polubinska,* Helmut Friess,‡ Gerhard M. Gahl,† Ulrich Frei,† and Achim Jörres[2†]

IL-17 is a newly discovered cytokine implicated in the regulation of hemopoiesis and inflammation. Because IL-17 production is restricted to activated T lymphocytes, the effects exerted by IL-17 may help one to understand the contribution of T cells to the inflammatory response. We investigated the role of IL-17 in leukocyte recruitment into the peritoneal cavity. Leukocyte infiltration in vivo was assessed in BALB/Cj mice. Effects of IL-17 on chemokine generation in vitro were examined in human peritoneal mesothelial cells (HPMC). Administration of IL-17 i.p. resulted in a selective recruitment of neutrophils into the peritoneum and increased levels of KC chemokine (murine homologue of human growth-related oncogene α (GROα). Pretreatment with anti-KC Ab significantly reduced the IL-17-driven neutrophil accumulation. Primary cultures of HPMC expressed IL-17 receptor mRNA. Exposure of HPMC to IL-17 led to a dose- and time-dependent induction of GROα mRNA and protein. Combination of IL-17 together with TNF-α resulted in an increased stability of GROα mRNA and synergistic release of GROα protein. Anti-IL-17 Ab blocked the effects of IL-17 in vitro and in vivo. IL-17 is capable of selectively recruiting neutrophils into the peritoneal cavity via the release of neutrophil-specific chemokines from the peritoneal mesothelium. *The Journal of Immunology,* **2000, 165: 5814–5821.**

Peritoneal macrophages are commonly viewed as the first line of defense against invading microorganisms (1, 2). The role of other leukocyte populations in peritoneal immunity is, however, less understood. In healthy individuals, 5–10% of peritoneal leukocytes are lymphocytes, with the vast majority belonging to the T lineage (3, 4). It has been suggested that the specific peritoneal microenvironment may affect T cell selection in the peritoneum (5). As compared with PBLs, the peritoneal $CD4^+$:$CD8^+$ ratio is inverted (4–7), and within these subsets ~70–90% cells exhibit the $CD45RO^+$ phenotype of memory cells (8). The substantial proportion of peritoneal T-cells have also been found to express either $CD8^+\alpha\alpha$ isoform or *RAG-1* mRNA transcripts (5, 8). The presence of these traits is believed to reflect thymus-independent T cell differentiation (9) and may support the concept of the peritoneal lymphoid tissue as an intestinal thymus (10, 11). A broad spectrum of effects exerted by lymphocyte-derived mediator IFN-γ (12) points to a significant role of lymphocytes in the inflammatory response. In this respect, increased levels of IFN-γ have been detected in the inflamed peritoneum, and this rise has been clearly attributed to peritoneal lymphocytes (13).

IL-17 is a newly identified T cell-specific cytokine (14). The human form of IL-17 is a ~20-kDa glycoprotein of 155 aa, the sequence of which exhibits a close homology to both cytotoxic T lymphocyte-associated Ag-8 (CTLA-8) and the open reading frame 13 of T-lymphotropic *Herpesvirus saimiri* (HVS-13) (15, 16). Expression of IL-17 has been detected almost exclusively in activated $CD4^+$ and $CD8^+$ T lymphocytes (predominantly of the memory $CD45RO^+$ subset) (15, 17–19). In sharp contrast, the specific IL-17R is widely distributed in most tissues and cell lines (20, 21). Accumulating evidence suggests that IL-17 may be an important mediator of the hemopoietic system (14, 22). It has been found to stimulate the production of IL-6, G-CSF, and LIF (17, 21, 23, 24)- cytokines with a known impact on hemopoietic progenitors. Moreover, the soluble form of IL-17R protein has been shown to inhibit mitogen-induced proliferation and IL-2 production in murine T cells (21). In contrast, IL-17 also appears to be involved in the inflammatory reaction. The expression of several genes associated with inflammation, including IL-1β, TNF-α, IL-6, IL-8, cyclooxygenase-2, NO synthase, and stromelysin, is up-regulated after stimulation with IL-17 (17, 23, 25–29). In addition, increased production of the complement component C3 in response to IL-17 has been detected in renal proximal tubule cells (28).

In this study, we set out to investigate the potential role of IL-17 in the peritoneal inflammatory response. We demonstrate that IL-17 selectively recruits neutrophils into the peritoneal cavity and that this effect is likely to be mediated via the release of neutrophil-specific chemokines from the peritoneal mesothelium.

Materials and Methods

Materials

All chemicals, unless otherwise stated, were purchased from Sigma-Aldrich Chemie (Deisenhofen, Germany). Tissue culture plastics were from

*Department of Pathophysiology, University Medical School, Poznan, Poland; †Department of Nephrology and Medical Intensive Care, Universitätsklinikum Charité, Campus Virchow-Klinikum, Humboldt-Universität zu Berlin, Berlin, Germany; and ‡Department of Visceral and Transplant Surgery, University of Bern, Inselspital, Bern, Switzerland

Received for publication February 23, 2000. Accepted for publication August 21, 2000.

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

[1] A.J. was supported by a grant from the Else Kröner-Fresenius Foundation (Bad Homburg, Germany).

[2] Address correspondence and reprint requests to Dr. Achim Jörres, Department of Nephrology and Medical Intensive Care, Universitätsklinikum Charité, Campus Virchow-Klinikum, Augustenburger Platz 1, D-13353 Berlin, Germany. E-mail address: achim.joerres@charite.de

0022-1767/00/$02.00

Falcon Becton Dickinson (Heidelberg, Germany). Recombinant human and murine cytokines and anti-cytokine Abs were obtained from R&D Systems (Wiesbaden, Germany). According to the manufacturer, the endotoxin concentration in the above materials was <0.1 ng/μg protein as measured by *Limulus* amebocyte lysate assay. All cytokine preparations were batched, stored at −70°C, and freshly thawed for each experiment. All media and buffers were of tissue culture grade with an endotoxin concentration of <0.1 ng/ml.

Animal studies

All in vivo experiments were performed using male BALB/Cj inbred mice weighing 25–30 g. BALB/CJ/Han/Imp mice were obtained from the Institute of Occupational Medicine (Lodz, Poland) and housed under 12-h light/dark cycles with free access to standard chow and water. All studies were performed according to the guidelines of the Committee for Animal Studies at University Medical School (Poznan, Poland).

Recombinant mouse IL-17 (rmIL-17) was diluted in sterile endotoxin-free PBS (Dulbecco's PBS, PAA Laboratories, Linz, Austria) and administered i.p. at a dose of 0.5 μg/mice in a total volume of 500 μl. The concentration of IL-17 to be applied was determined in preliminary dose-response experiments which showed that 0.5 μg IL-17 consistently produced a significant influx of neutrophils (data not shown). Control animals received an equivalent volume of PBS alone. For the procedure the animals were placed under brief ether anesthesia and then allowed to recover. In separate experiments, IL-17 (0.5 μg) was preincubated with monoclonal anti-mouse IL-17 neutralizing Ab (10 μg) at 37°C for 15 min before i.p. administration in 500 μl PBS. In further studies, the animals were first given i.p. injection of either monoclonal anti-mouse KC neutralizing Ab (10 μg/mouse in 200 μl PBS) or PBS alone, and after 15 min they received i.p. either IL-17 (0.5 μg in 300 μl PBS) or PBS alone. In an additional set of experiments, recombinant mouse KC was administered i.p. in 500 μl PBS.

At designated time points, the animals were anesthetized, sacrificed by bleeding, and injected i.p. with 2.5 ml PBS containing 3 mM EDTA (PBS/EDTA) (30). The peritoneal cavity was then opened, and the lavage fluid was carefully collected. Cell pellets were resuspended in PBS/EDTA, and total cell counts were determined in a hemocytometer using Türk's solution. Differential cell counting was performed on cytospin preparations stained with May-Grünwald-Giemsa using a QCA staining kit (Quimica Clinica Aplicada, Amposta, Spain). Aliquots of cell-free peritoneal lavage fluids and sera were stored at −70°C until further analysis for cytokines.

Peritoneal mesothelial cell culture

Human peritoneal mesothelial cells (HPMC)[3] were isolated from the specimens of omentum obtained from consenting patients undergoing elective abdominal surgery. Cells were isolated and characterized as described in detail elsewhere (31, 32). Cells were propagated in Earle's buffered M199 culture medium (Seromed, Biochrom, Berlin, Germany) supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), hydrocortisone (0.4 μg/ml), and 10% v/v FCS (Life Technologies, Eggenstein, Germany). Cell cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere.

All experiments were performed using cells derived from at least six separate donors, and from the first or second passage to minimize the number of senescent cells which appeared from the third passage onwards (31).

Effect of IL-17 on the production of growth-related oncogene product α (GROα) by human peritoneal mesothelial cells

HPMC were grown to confluence and rendered quiescent by serum deprivation for 48 h before stimulation. Preliminary experiments had demonstrated that under these conditions cells could be maintained for at least up to 120 h without any significant loss of viability (as assessed by intracellular ATP concentrations). HPMC were exposed to recombinant human IL-17 at doses ranging from 0.01 to 100 ng/ml. In some experiments, cells were exposed to IL-17 in the presence or absence of TNF-α. In the inhibition studies, HPMC were pretreated with transcription (actinomycin D) or translation (cycloheximide) inhibitors for 45 and 120 min, respectively, and then stimulated with IL-17. The doses of inhibitors used did not impair cell viability. In separate experiments, IL-17 preparations were first preincubated with either anti-human IL-17 polyclonal neutralizing Ab or the equivalent dose of control IgG of the same class, and then applied to HPMC cultures.

At designated time intervals, the cell supernatants were removed, centrifuged at 12,000 × *g* to remove any cellular debris, and stored at −70°C until assayed. Cell monolayers were washed with PBS and solubilized with 0.1 N NaOH. Total cellular protein was then analyzed using Bradford method with Bio-Rad Protein Assay Dye Reagent (Bio-Rad Laboratories, Munich, Germany) and BSA as the standard. Repeated cell counts revealed that 1 μg HPMC protein corresponded to (mean ± SD) $2.1 \pm 1.0 \times 10^3$ cells ($n = 16$). All data for GROα secretion were expressed as picograms per microgram cellular protein.

Cytokine measurements

Concentrations of mouse KC and macrophage inflammatory protein-2 (MIP-2) in serum and peritoneal lavage fluid were determined using Quantikine Mouse Immunoassays (R&D Systems) with sensitivities of 2 pg/ml for KC and 1.5 pg/ml for MIP-2. GROα levels in supernatants from HPMC cultures were measured using the Quantikine Human GROα Immunoassay (R&D Systems) with a sensitivity of 5.0 pg/ml.

RNA isolation and analysis

Total RNA from HPMC cultures was extracted with the RNA Isolator (Genosys Biotechnologies, Cambridge, U.K.) and purified according to the manufacturer's protocol. Expression of IL-17R and of IL-17-induced GROα mRNA was assessed using reverse transcription-PCR or Northern blot analysis as described below.

Reverse transcription and PCR

One microgram of total RNA was reverse transcribed into cDNA with random hexamer primers, as previously described (33). PCR amplification was performed in a total volume of 50 μl consisting of 2 μl reverse transcription product and 48 μl of the reaction master mix. The master mix contained 36.25 μl H_2O, 2.5 μl sense and antisense primers (20 μM each), 4 μl dNTPs, 5 μl 10× PCR buffer (100 mM Tris, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), and 0.25 μl *Taq* polymerase (1.25 U, Amplitaq; Perkin-Elmer Cetus, Weiterstadt, Germany). The amplification was conducted on the Perkin-Elmer 480 Thermocycler (Perkin-Elmer Cetus, Applied Biosystems). Specific oligonucleotide primer pairs were synthesized by TIB MolBiol SyntheseLabor (Berlin, Germany). The primer sequences were as shown in Table I.

The reaction for αβ-actin and GROα amplicons began with a 3-min denaturation step at 94°C and was followed by 27 (GROα), 30 (α-actin), or 33 (β-actin) cycles of denaturation at 94°C for 40 s, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The final cycle was 94°C for 40 s and 60°C for 10 min. Preliminary experiments had determined that under these conditions PCR products were generated during the exponential phase of amplification. The protocol of PCR amplification with primers for IL-17R was similar except that annealing temperature was 60°C, samples were amplified for 35 cycles, and the final extension was at 72°C for 8 min. PCR products were separated by electrophoresis in 3% agarose gels (FMC Bioproducts, Biozym Diagnostic, Hess Oldendorf, Germany), stained with ethidium bromide (1 μg/ml) and visualized under UV transillumination. Expression of target mRNAs was assessed by comparison with the expression of the "housekeeping" genes of α- or β-actin in the same sample. The bands corresponding to the intended products were analyzed using Scanpack 14.1A27 software (Biometra, Göttingen, Germany).

Northern blot analysis

Twenty micrograms total RNA were size-fractionated on 1.2% agarose, 1.8 M formaldehyde gels and stained with ethidium bromide for verification of RNA integrity and loading equivalency (38–40). The RNA was electrotransferred onto nylon membranes (Gene Screen, DuPont, Boston, MA) and cross-linked by UV irradiation. The filters were then prehybridized, hybridized, and washed under conditions appropriate for the α-^{32}P-labeled GROα and 7S cDNA probes, as previously described in detail (38–40). Prehybridization of the membranes was conducted for 4–8 h at 42°C in a buffer containing 30% formamide, 1% SDS, 0.75 M NaCl, 5 mM EDTA, 5× Denhardt's solution, 100 μg/ml salmon sperm DNA, 10% dextran sulfate, and 50 mM sodium phosphate buffer, pH 7.4. Hybridization was conducted at 42°C for 18 h with either 10^6 cpm/ml of the ^{32}P-labeled GROα probe or 10^5 cpm/ml of the ^{32}P-labeled 7S cDNA probe. After the hybridization, the blots were washed under stringent conditions with two rinses in 2× SSC at 50°C and three washes (20 min each) in 0.2× SSC/2% SDS at 55°C. Blots were then exposed to Fuji x-ray films with intensifying screens (DuPont) at −80°C, and the intensity of the radiographic bands

[3] Abbreviations used in this paper: HPMC, human peritoneal mesothelial cells; GROα, growth-related oncogene α; MIP-2, macrophage inflammatory protein-2; PMN, polymorphonuclear leukocyte.

was quantified by video image analysis (Image-Pro plus, Media Cybernetics, Silver Spring, MD), as previously reported (38, 41). The ratio between GROα and corresponding 7S signal was calculated for each sample.

Probe for Northern blot analysis

GROα DNA probe used in Northern blot analysis consisted of a 231-bp fragment corresponding to positions 452–682 of the GROα mRNA. The probe was designed using nucleotide sequence of the GROα gene as published by Baker et al. (42). The fragment was cloned by reverse transcribing human normal pancreas RNA, amplifying the cDNA obtained by PCR, and ligating the amplicon into the pGEM-T Easy Vector (Promega, Biotechnology, Madison, WI). The 7S DNA probe consisted of a 212-bp fragment of the 7S RNA which was cloned as described above. This probe was used to verify equivalent RNA loading in the Northern blot experiments (38, 40). Authenticity of GROα and 7S fragments was confirmed by sequencing using the dye terminator method (ABI 373A, Perkin-Elmer, Rotkreuz, Switzerland). For Northern blot analysis, the GROα and 7S DNA probes were radiolabeled with [α-^{32}P]dCTP (DuPont International, Regensdorf, Switzerland) using a random primer labeling system (NEN Life Science Products, Boston, MA).

Stability of IL-17-induced GROα mRNA

The inherent stability of mesothelial cell GROα mRNA was assessed by measuring the rate of GROα mRNA degradation in the presence of the transcription inhibitor actinomycin D. HPMC were stimulated with IL-17 (50 ng/ml) in the presence or absence of TNF-α (1 ng/ml) for 2 h. After that, cells were washed and pulsed with actinomycin D (5 μg/ml). At defined time intervals, the total RNA was extracted, reverse transcribed into cDNA, and PCR amplified for GROα and α-actin as described above.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 3.00 software (GraphPad Software, San Diego, CA). Multiple comparisons of paired data were made with nonparametric repeated measures ANOVA with Friedman modification. Unpaired data derived from animal studies were analyzed using Mann-Whitney *U* or Kruskal-Wallis tests, when appropriate. A p value of <0.05 was considered significant. All data are presented as means ± SEM.

Results

IL-17 induces i.p. neutrophil infiltration

Injection of IL-17 i.p. in experimental animals resulted in a time-dependent increase in the total number of cells in the peritoneal cavity (Fig. 1). The maximal effect was observed 4 h after the administration of IL-17 when the accumulation of cells was 2.6-fold above the numbers detected in control mice. Differential cell counting revealed that this increase could be accounted for by a substantial rise in the number of polymorphonuclear neutrophils (PMN, Table II, Fig. 2). In untreated mice (no i.p. injections, lavage only, $n = 9$), PMN constituted merely 1.1 ± 0.4% of the peritoneal cell population. In control animals, the procedure of i.p. injection and/or PBS itself produced a small nonspecific increase in the number of PMN. However, the influx of PMN triggered by IL-17 was significantly above these background levels (data not shown). During the time frame studied, the absolute number of cells from other populations did not differ significantly from those detected in control and untreated animals (Table II). Administration of IL-17 together with anti-IL-17 neutralizing Ab reduced the specific IL-17-mediated PMN influx by 61.2 ± 9.5% within 4 h.

IL-17 stimulates i.p. generation of KC chemokine

Administration of IL-17 produced a massive increase in the i.p. concentrations of KC chemokine, a murine analogue of human GROα (Ref. 43 and Fig. 2). The level of KC increased rapidly within 1 h and by 4 h returned to basal values. Comparison of KC concentrations in sera and lavage fluids indicated that KC released in response to IL-17 was of the local i.p. origin. At the 1-h time point, the mean concentration of KC in the lavage fluid was 3401 ± 592 pg/ml compared with 264 ± 50 pg/ml in serum ($n = 6$). The mean serum KC level in untreated mice was 150 ± 48 pg/ml ($n = 8$) and corresponded to the values detected by the manufacturer of the mouse KC immunoassay (R&D Systems). In addition, IL-17 triggered a rapid i.p. release of neutrophil chemoattractant MIP-2. Within 1 h, MIP-2 concentration in the lavage fluid rose from 14 ± 5 pg/ml to 1250 ± 116 pg/ml, compared with plasma levels of 45 ± 6 pg/ml ($n = 6$–9).

Neutralization of KC reduces IL-17-stimulated neutrophil recruitment

The observation that IL-17-induced PMN influx was preceded by a rapid increase in KC levels (Fig. 2) suggested that the effect could have been mediated by KC, a powerful neutrophil chemoattractant (43). Treatment i.p. with recombinant KC resulted in a dose-dependent and selective increase in PMN recruitment (data not shown). The dose of 0.5 μg KC/animal produced a 7.5 ± 0.7-fold increase in PMN accumulation within 4 h. Furthermore, administration of anti-KC neutralizing Ab before IL-17 injection reduced the IL-17-specific PMN infiltration within 4 h by 67.2 ± 7.9%, although it had no significant effect on basal PMN influx triggered by the injection of PBS vehicle (Fig. 3).

Peritoneal mesothelial cells express IL-17R

RT-PCR analysis using primers specific for either the extracellular or intracellular domain of IL-17R revealed that HPMC expressed IL-17R mRNA. Constitutive expression of IL-17R gene transcripts was detected in all primary cultures of HPMC examined (Fig. 4).

Table I. *Primer sequences for specific oligonucleotide primer pairs*

Primer Pairs	Sequence[a]	Length (bp)	Ref.
GROα	F: 5′-ACTCAAGAATGGGCGGAAAG-3′ R: 5′-TGGCATGTTGCAGGCTCCT-3′	468	34
IL-17R extracellular domain	F: 5′-CTAAACTGCACGGTCAAGAAT-3′ R: 5′-ATGAACCAGTACACCCAC-3′	833	20
IL-17R intracellular domain	F: 5′-ATGGACAGGTTCGAGGAG-3′ R: 5′-TTCACGATGCCGGTTCCC-3′	276	35
α-Actin	F: 5′-GGAGCAATGATCTTGATCTT-3′ R: 5′-TCCTGAGGTACGGGTCCTTCC-3′	204	36
β-Actin	F: 5′-ATCCCCCAAAGTTCACAA-3′ R: 5′-CTGGGCCATTCTCCTTAG-3′	147	37

[a] F, forward; R, reverse.

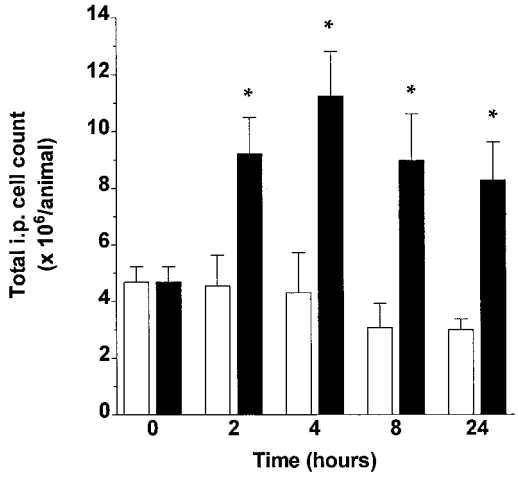

FIGURE 1. Time course of changes in total cell number in peritoneal cavity in response to IL-17. Mice received either 0.5 μg IL-17 (■) or an equivalent volume of PBS vehicle (controls, □). At each time point, the peritoneal lavage fluid was obtained from four to nine animals. *, Statistically significant difference compared with the values obtained from the controls at the same time point.

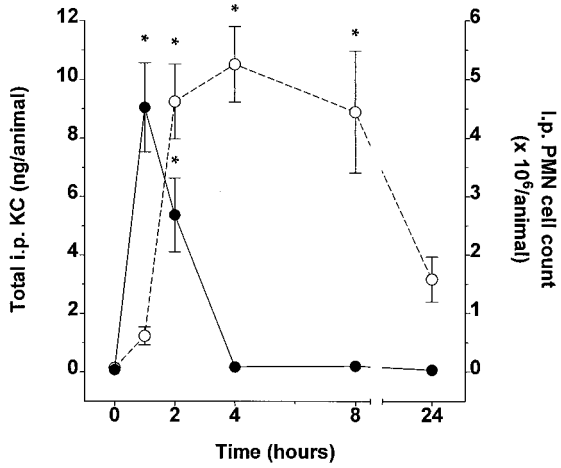

FIGURE 2. Changes in i.p. KC and neutrophil number following the administration of IL-17. The total amount of KC (●) and the absolute number of PMN (○) in the peritoneal cavity was determined in mice injected with 0.5 μg rmIL-17. Data were obtained from six to nine animals per time point. *, Significant difference compared with values obtained before the injection of IL-17.

IL-17 stimulates GROα production by peritoneal mesothelial cells

HPMC released GROα constitutively. Exposure of HPMC to a recombinant form of human IL-17 resulted in a time- and dose-dependent increase in GROα generation. With IL-17 at a dose of 50 ng/ml, this increase became significant above control levels after 6 h of incubation and was followed by large increments during the next 18 h and a plateau by 24 h (Fig. 5). Statistically significant increase in GROα secretion was achieved with IL-17 at a dose of 1 ng/ml and above (Fig. 6). Administration of IL-17 in the presence of anti-IL-17 neutralizing Ab reduced IL-17-stimulated GROα production from 825.9 ± 97.3 to 131.8 ± 35.9 pg/μg cell protein ($n = 7$, $p < 0.05$), approximately to the baseline release of GROα (107.6 ± 33.9 pg/μg cell protein). In contrast, control Ab of the same class did not affect the stimulatory activity of IL-17 (882.0 ± 115.0 pg/μg cell protein).

Transcription and translation inhibitors reduce IL-17-stimulated GROα release

Preexposure of HPMC to actinomycin D for 45 min at 37°C resulted in a dose-dependent decrease in IL-17-stimulated but not in constitutive GROα secretion. Maximal inhibition was achieved with the dose of 1 μg/ml, which reduced IL-17-driven GROα release by 90.1 ± 6.8% (687.3 ± 116.8 vs 68.1 ± 46.8 pg/μg cell protein) to the level detected in unstimulated cells ($n = 5$, $p < 0.01$). Generation of GROα in HPMC stimulated with IL-17 could also be inhibited by cycloheximide. At the highest nontoxic dose of cycloheximide tested (50 μg/ml) GROα release was reduced by 51.4 ± 6.1% (747.3 ± 105.4 vs 363.1 ± 45.5 pg/μg cell protein, $n = 7$, $p < 0.01$).

Exposure to IL-17 induces GROα mRNA in peritoneal mesothelial cells

Stimulation of HPMC with IL-17 induced a time- and dose-dependent accumulation of GROα mRNA as demonstrated by Northern blot analysis (Fig. 7). Unstimulated cells expressed very faint signals for GROα mRNA. After treatment with IL-17, the GROα mRNA expression was rapidly up-regulated within 1 h. Increased expression of GROα mRNA was detected in cells stimulated with a dose of IL-17 as low as 0.1 ng/ml.

TNF-α superinduces IL-17-driven GROα synthesis by stabilizing GROα mRNA

Exposure of HPMC to TNF-α increased GROα release in a time- and dose-dependent manner (data not shown). Combination of TNF-α together with IL-17 triggered GROα production above the levels generated by each stimulus alone and significantly above the calculated additive value (Fig. 8). This synergistic effect became

Table II. *Changes in number of cells in the peritoneal cavity following the administration of IL-17*[a]

	i.p. Cell Number (× 10^3/animal)						
Time (h)	Neutrophils	Macrophages	Monocytes	Lymphocytes	Mast cells	Eosinophils	Mesothelial cells
0	68 ± 28	1955 ± 294	265 ± 197	3167 ± 403	77 ± 22	0	102 ± 96
1	616 ± 153	1009 ± 89	160 ± 80	2834 ± 489	40 ± 20	0	0
2	4625 ± 637***	1531 ± 469	288 ± 157	2570 ± 418	99 ± 41	0	8 ± 7
4	5147 ± 593***	2256 ± 373	746 ± 318	2902 ± 409	26 ± 15	0	34 ± 24
8	4446 ± 1038**	1064 ± 449	386 ± 177	2954 ± 789	44 ± 36	0	0
24	1584 ± 385	1663 ± 269	808 ± 345	4090 ± 904	95 ± 36	0	0

[a] Mice were injected i.p. with 0.5 μg rmIL-17. At designated time intervals, the peritoneal lavage and differential cell counting were performed as described in *Materials and Methods*. Data were derived from 6–9 animals for each time point and were expressed as absolute numbers of cells in the peritoneum. Asterisks represent a statistically significant difference compared with values obtained before the injection of IL-17.

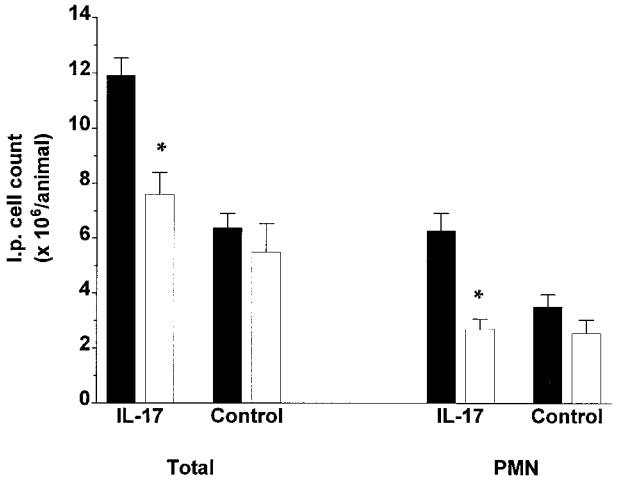

FIGURE 3. Effect of anti-KC Ab on IL-17-induced neutrophil accumulation in the peritoneal cavity. Animals were pretreated with either anti-KC neutralizing Ab or PBS vehicle (controls), and then injected with either rmIL-17 or PBS, as described in *Materials and Methods*. The number of PMN in the peritoneal cavity was determined 4 h later. Data were obtained from seven animals per condition. *, Significant difference compared with values obtained from the controls.

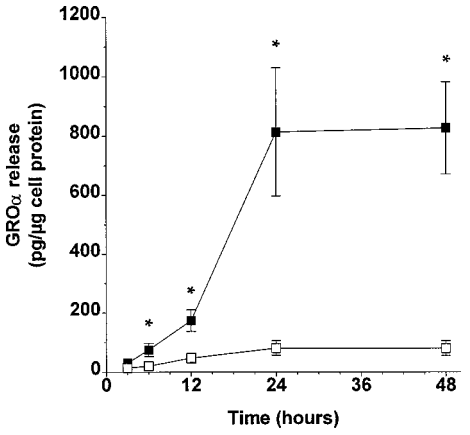

FIGURE 5. Time course of GROα release from HPMC in response to IL-17. Quiescent HPMC were exposed to either control medium (□) or 50 ng/ml recombinant human IL-17 (■). At designated time intervals, the supernatants were collected and assayed for GROα. Data are presented as mean values (±SEM) obtained from six experiments with cells isolated from separate donors. *, Statistically significant difference compared with the unstimulated control at the same time point.

evident when TNF-α (1 ng/ml) was combined with 0.1 ng/ml IL-17, and maximal synergy was obtained at the highest dose of IL-17 tested (100 ng/ml). GROα release under these conditions was 2.2 ± 0.2-fold above the predicted additive value. Actinomycin D chase experiments revealed that in HPMC treated with a combination of IL-17 and TNF-α the degradation of GROα mRNA was delayed compared with cells treated with IL-17 alone (Fig. 9).

Discussion

Increasing evidence suggests that IL-17, acting either directly or indirectly, may significantly affect neutrophil maturation and function. Initial observations came from Fossiez et al. (17), who had found that IL-17-treated fibroblasts produced G-CSF and were capable of promoting differentiation of $CD34^+$ hemopoietic progenitors toward neutrophils. Subsequently, Schwarzenberger et al. demonstrated that adenovirus-mediated overexpression of IL-17 in mice resulted in a massive peripheral neutrophilia associated with increased levels of G-CSF and marked stimulation of splenic granulopoiesis (44). Additional experiments revealed that in several cell systems IL-17 induced the release of neutrophil-specific chemokines (17, 29, 35, 45). With the present study, we extend these observation and demonstrate that IL-17 possesses a significant potential to recruit neutrophils into the peritoneum.

Administration of IL-17 i.p. resulted in a massive and selective influx of neutrophils. Neutrophil population appeared to have been targeted specifically because the trafficking of other leukocyte subsets was not significantly affected. Similar effects of IL-17 were observed in the rat airways where intratracheal IL-17 instillation induced selective accumulation of neutrophils in the bronchoalveolar lavage fluid (45, 46). The specificity of these effects was confirmed by the inhibition with anti-IL-17 neutralizing Ab. The fact that this inhibition was incomplete (~60%) could be explained by the limited neutralizing capacity of the Ab under in vivo conditions in comparison with the dose of IL-17 used.

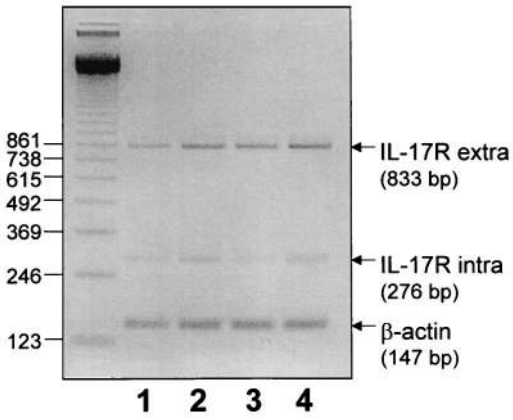

FIGURE 4. Expression of IL-17R mRNA in HPMC. Total RNA was extracted from primary cultures of human peritoneal mesothelial cells isolated from four donors (*lanes 1–4*). After reverse transcription and PCR amplification with primers specific for intra- and extracellular domain of IL-17R mRNA, the PCR products were resolved on ethidium bromide-stained 3% agarose gels.

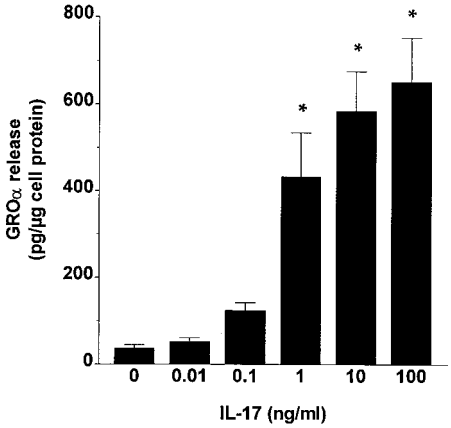

FIGURE 6. Dose effect of IL-17 on GROα secretion by HPMC. Quiescent HPMC were exposed to increasing doses of IL-17 for 24 h. Data represent the mean (±SEM) of seven experiments with HPMC isolated from different donors. *, Statistically significant difference compared with the control.

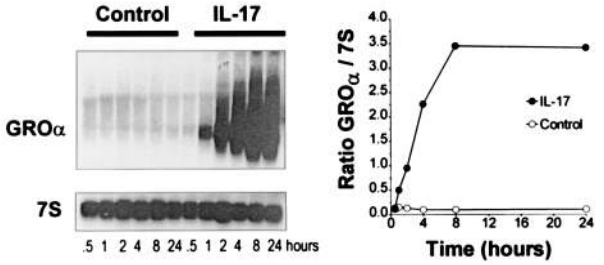

FIGURE 7. Expression of GROα mRNA in HPMC treated with IL-17. Quiescent HPMC were exposed to IL-17 (50 ng/ml) for the time periods indicated. Total RNA was extracted and subjected to Northern blot hybridization with probes for GROα and 7S, as described in *Materials and Methods*. After densitometric analysis, the data were expressed as GROα:7S ratios.

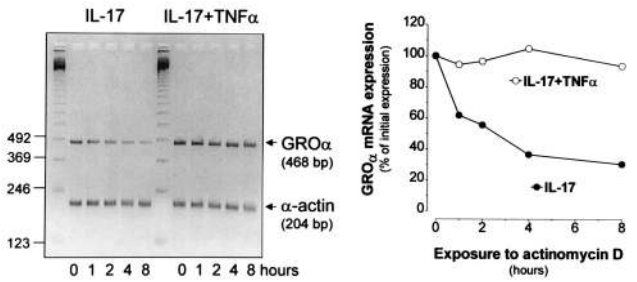

FIGURE 9. Effect of TNF-α on the stability of IL-17-induced GROα mRNA. Quiescent HPMC were stimulated with either IL-17 (50 ng/ml; ○) or the combination of IL-17 (50 ng/ml) and TNF-α (1 ng/ml; ●) for 2 h and then treated with actinomycin D (5 μg/ml). Total RNA was extracted at the time points indicated and analyzed by RT-PCR. PCR products were separated on 3% agarose gels stained with ethidium bromide (*A*). After densitometric analysis, the GROα:α-actin ratios were calculated, and the results were expressed as a percentage of the value obtained at time zero (*B*).

Because IL-17 has been shown to have no direct effect on neutrophil chemotaxis in vitro (45), we hypothesized that PMN accumulation in response to IL-17 could have been mediated by the induction of chemokines. Analysis of the peritoneal fluid revealed that IL-17 induced a rapid rise in i.p. levels of KC which preceded the influx of PMN. The similar time course of KC induction has been observed in various models of murine peritonitis (30, 47). KC is a chemokine with a powerful chemotactic activity toward neutrophils (48) and, indeed, the i.p. injection of recombinant KC triggered a massive accumulation of PMN in the peritoneal cavity. Furthermore, when IL-17-receiving animals were pretreated with anti-KC neutralizing Ab, the IL-17-mediated PMN recruitment was reduced by >60%. Again, the magnitude of inhibition observed under these conditions could be influenced by the neutralizing capacity of the Ab used. However, it is also possible that the remaining chemotactic activity could be attributed to other chemokines induced by IL-17. Indeed, we found that IL-17 also produced a significant rise in i.p. MIP-2 levels. In this respect, Laan et al. (45) have convincingly documented the role of MIP-2 as a mediator of IL-17-induced leukocyte trafficking in the rat, and Walley et al. (49) have demonstrated the importance of MIP-2 in the pathogenesis of cecal ligation and puncture model of peritonitis. MIP-2 is a chemokine with no exact human homologue but closely related to either IL-8 (50) or GROβγ (43, 51).

The observation that the levels of KC in the peritoneum were much higher than those detected in serum suggested the local origin of KC. One possible source could be the peritoneal macrophage; however, in a recent study Ajuebor et al. (47) have demonstrated that in an LPS model of peritonitis in mice the removal of peritoneal macrophages and monocytes did not diminish the peritoneal generation of KC. We have therefore concentrated on peritoneal mesothelial cells. The peritoneal mesothelium is a recognized source of chemotactic activity in the peritoneum (32, 52–54), and it has been demonstrated that on appropriate stimulation human mesothelial cells are capable of generating GROα, a homologue of KC (55). The presence of IL-17R mRNA in HPMC was demonstrated by RT-PCR and confirmed the ubiquitous nature of IL-17R distribution (20, 21). Exposure of quiescent HPMC to IL-17 led to a significant time- and dose-dependent increase in the secretion of GROα. The range of IL-17 doses that triggered this effect corresponded to those that had been shown to stimulate the release of cytokines in other in vitro systems (17, 25, 26, 28, 29, 35, 45, 56). The IL-17-driven GROα release could be inhibited in a dose-dependent manner by the pretreatment of HPMC with both transcription and translation inhibitors which suggested that IL-17 stimulated de novo GROα synthesis. Northern blot analysis confirmed that exposure of HPMC to IL-17 resulted in a rapid upregulation of GROα mRNA. Using a rat intestinal epithelial cell line, Awane et al. (29) have recently analyzed the IL-17-activated

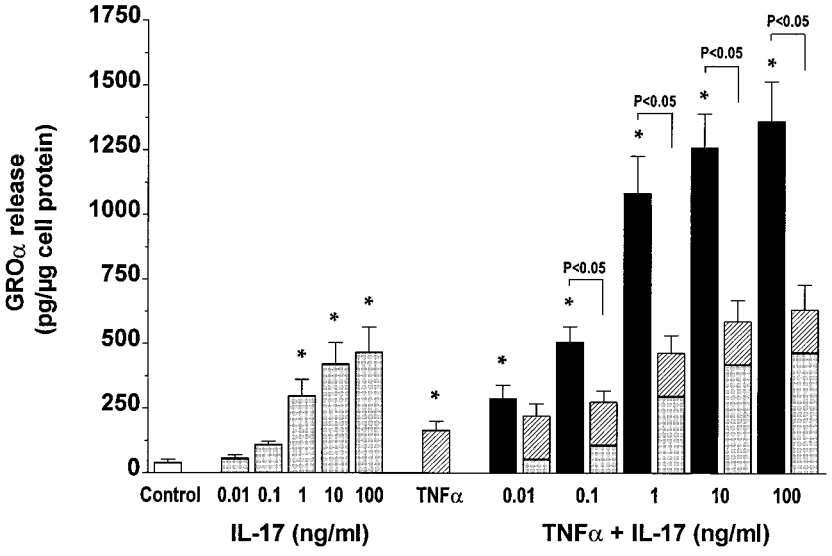

FIGURE 8. Effect of combined IL-17 and TNF-α stimulation on GROα production by HPMC. Quiescent cells were exposed to control medium or TNF-α (1 ng/ml) in the presence of increasing doses of IL-17 (0.01–100 ng/ml). After a 24 h incubation, the supernatants were assayed for GROα. Data were derived from seven experiments with HPMC isolated from different donors. □, control; ▨, TNF-α; ▩, IL-17; ■, IL-17 + TNFα. Composite bars represent expected additive values. *, Statistically significant difference compared with the control.

signal transduction pathway leading to the induction of CINC, a C-X-C chemokine related to human GROα. In this system, IL-17 has been shown to induce the activity of NF-κB-dependent CINC promoter via the pathway regulated by TNFR-associated factor-6 and NF-κB-inducing kinase. IL-17-induced NF-κB activity has also been demonstrated in macrophages (25), chondrocytes (26), and fibroblasts (21).

We have found that IL-17-induced generation of GROα could be synergistically augmented in the presence of TNF-α. This effect was at least partially related to the stabilization of GRO mRNA. Because IL-17 has been demonstrated to stimulate the release of TNF-α from macrophages (25), one may imagine that by acting simultaneously on mesothelial cell and macrophages in the peritoneal cavity in vivo lymphocyte-derived IL-17 amplifies the generation of GROα and increases the transperitoneal chemotactic gradient for neutrophils. In this respect, it has been demonstrated that truncated form of GROα, which acts as a C-X-C chemokine receptor antagonist, is capable of inhibiting leukocyte recruitment into the peritoneal cavity (57). In other cell systems, IL-17 has been shown to synergize with TNF-α in the production of IL-8 (35, 45), IL-6, and GM-CSF (17). The potential synergy between IL-17 and TNF-α under in vivo conditions is currently being investigated.

The exact role of IL-17 in human physiology and pathology remains to be determined. Available data suggest that IL-17 may promote cartilage destruction in various forms of arthritis (23, 26, 56, 58, 59) and mediate alloimmune reactivity and organ allograft rejection (28, 60). Our findings demonstrate that IL-17 may act as a potent and selective inducer of neutrophil chemotaxis. In the setting of the peritoneal cavity, this effect appears to be mediated through the stimulation of GROα release from peritoneal mesothelial cells. These properties of IL-17 provide further evidence that it can be classified as a proinflammatory cytokine (22). Because IL-17-producing CD45RO$^+$ cells predominate among i.p. lymphocytes, IL-17 may have a significant role to play in the peritoneal inflammatory response. Our findings also add to the understanding of mesothelial cell biology and point to the importance of lymphocyte-mesothelial interactions in peritonitis.

References

1. Brulez, H. F., and H. A. Verbrugh. 1995. First-line defense mechanisms in the peritoneal cavity during peritoneal dialysis. *Peritoneal Dial. Int. 15:S24.*
2. Holmes, C. J. 1994. Peritoneal host defense mechanisms in peritoneal dialysis. *Kidney Int. Suppl. 48:S58.*
3. Rapoport, J., M. J. Hausmann, and C. Chaimovitz. 1999. The peritoneal immune system and continuous ambulatory peritoneal dialysis. *Nephron 81:373.*
4. Lewis, S. L., S. G. Kutvirt, C. L. Cooper, P. N. Bonner, and C. J. Holmes. 1993. Characteristics of peripheral and peritoneal lymphocytes from continuous ambulatory peritoneal dialysis patients. *Peritoneal Dial. Int. 13(Suppl. 2):S273.*
5. Hartman, J., V. Maassen, P. Rieber, and H. Fricke. 1995. T lymphocytes from normal human peritoneum are phenotypically different from their counterparts in peripheral blood and CD3- lymphocyte subsets contain mRNA for the recombination activating gene RAG-1. *Eur. J. Immunol. 25:2626.*
6. Kubicka, U., W. L. Olszewski, W. Tarnowski, K. Bielecki, A. Ziolkowska, and Z. Wierzbicki. 1996. Normal human immune peritoneal cells: subpopulations and functional characteristics. *Scand. J. Immunol. 44:157.*
7. Betjes, M. G., C. W. Tuk, D. G. Struijk, R. T. Krediet, L. Arisz, E. C. Hoefsmit, and R. H. Beelen. 1993. Immuno-effector characteristics of peritoneal cells during CAPD treatment: a longitudinal study. *Kidney Int. 43:641.*
8. Fricke, H., J. Hartmann, T. Sitter, R. Steldinger, P. Rieber, and H. Schiffl. 1996. Continuous ambulatory peritoneal dialysis impairs T lymphocyte selection in the peritoneum. *Kidney Int. 49:1386.*
9. Poussier, P., and M. Julius. 1994. Thymus independent T cell development and selection in the intestinal epithelium. *Annu. Rev. Immunol. 12:521.*
10. Koten, J. W., and W. den Otter. 1991. Are omental milky spots an intestinal thymus? *Lancet 338:1189.*
11. Heel, K. A., and J. C. Hall. 1996. Peritoneal defences and peritoneum-associated lymphoid tissue. *Br. J. Surg. 83:1031.*
12. Boehm, U., T. Klamp, M. Groot, and J. C. Howard. 1997. Cellular responses to interferon-γ. *Annu. Rev. Immunol. 15:749.*
13. Dasgupta, M. K., M. Larabie, and P. F. Halloran. 1994. Interferon-gamma levels in peritoneal dialysis effluents: relation to peritonitis. *Kidney Int. 46:475.*
14. Fossiez, F., J. Banchereau, R. Murray, C. van Kooten, P. Garrone, and S. Lebecque. 1998. Interleukin-17. *Int. Rev. Immunol. 16:541.*
15. Yao, Z., S. L. Painter, W. C. Fanslow, D. Ulrich, B. M. Macduff, M. K. Spriggs, and R. J. Armitage. 1995. Human IL-17: a novel cytokine derived from T cells. *J. Immunol. 155:5483.*
16. Kennedy, J., D. L. Rossi, S. M. Zurawski, F. Vega, R. A. Kastelein, J. L. Wagner, C. H. Hannum, and A. Zlotnik. 1996. Mouse IL-17: a cytokine preferentially expressed by $\alpha\beta$ TCR$^+$CD4$^-$CD8$^-$ T cells. *J. Interferon Cytokine Res. 16:611.*
17. Fossiez, F., O. Djossou, P. Chomarat, R. L. Flores, Y. S. Ait, C. Maat, J. J. Pin, P. Garrone, E. Garcia, S. Saeland, et al. 1996. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med. 183:2593.*
18. Shin, H. C., N. Benbernou, H. Fekkar, S. Esnault, and M. Guenounou. 1998. Regulation of IL-17, IFN-γ and IL-10 in human CD8$^+$ T cells by cyclic AMP-dependent signal transduction pathway. *Cytokine 10:841.*
19. Shin, H. C., N. Benbernou, S. Esnault, and M. Guenounou. 1999. Expression of IL-17 in human memory CD45RO$^+$ T lymphocytes and its regulation by protein kinase A pathway. *Cytokine 11:257.*
20. Yao, Z., M. K. Spriggs, J. M. Derry, L. Strockbine, L. S. Park, T. VandenBos, J. D. Zappone, S. L. Painter, and R. J. Armitage. 1997. Molecular characterization of the human interleukin (IL)-17 receptor. *Cytokine 9:794.*
21. Yao, Z., W. C. Fanslow, M. F. Seldin, A. M. Rousseau, S. L. Painter, M. R. Comeau, J. I. Cohen, and M. K. Spriggs. 1995. *Herpesvirus saimiri* encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity 3:811.*
22. Spriggs, M. K. 1997. Interleukin-17 and its receptor. *J. Clin. Immunol. 17:366.*
23. Chabaud, M., F. Fossiez, J. L. Taupin, and P. Miossec. 1998. Enhancing effect of IL-17 on IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synoviocytes and its regulation by Th2 cytokines. *J. Immunol. 161:409.*
24. Cai, X. Y., J. Gommoll-CP, L. Justice, S. K. Narula, and J. S. Fine. 1998. Regulation of granulocyte colony-stimulating factor gene expression by interleukin-17. *Immunol. Lett. 62:51.*
25. Jovanovic, D. V., J. A. Di Battista, P. J. Martel, F. C. Jolicoeur, Y. He, M. Zhang, F. Mineau, and J. P. Pelletier. 1998. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-β and TNF-α, by human macrophages. *J. Immunol. 160:3513.*
26. Shalom, B. T., J. Quach, and M. Lotz. 1998. Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NF-κB. *J. Biol. Chem. 273:27467.*
27. Teunissen, M. B., C. W. Koomen, M. R. de Waal, E. A. Wierenga, and J. D. Bos. 1998. Interleukin-17 and interferon-γ synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J. Invest. Dermatol. 111:645.*
28. van Kooten, C., J. G. Boonstra, M. E. Paape, F. Fossiez, J. Banchereau, S. Lebecque, J. A. Bruijn, J. W. De Fijter, L. A. Van Es, and M. R. Daha. 1998. Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. *J. Am. Soc. Nephrol. 9:1526.*
29. Awane, M., P. G. Andres, D. J. Li, and H. C. Reinecker. 1999. NF-κB-inducing kinase is a common mediator of IL-17-, TNF-α-, and IL-1β-induced chemokine promoter activation in intestinal epithelial cells. *J. Immunol. 162:5337.*
30. Ajuebor, M. N., R. J. Flower, R. Hannon, M. Christie, K. Bowers, A. Verity, and M. Perretti. 1998. Endogenous monocyte chemoattractant protein-1 recruits monocytes in the zymosan peritonitis model. *J. Leukocyte Biol. 63:108.*
31. Stylianou, E., L. A. Jenner, M. Davies, G. A. Coles, and J. D. Williams. 1990. Isolation, culture and characterization of human peritoneal mesothelial cells. *Kidney Int. 37:1563.*
32. Topley, N., Z. Brown, A. Jörres, J. Westwick, M. Davies, G. A. Coles, and J. D. Williams. 1993. Human peritoneal mesothelial cells synthesize interleukin-8: synergistic induction by interleukin-1β and tumor necrosis factor-α. *Am. J. Pathol. 142:1876.*
33. Jörres, A., H. Dinter, N. Topley, G. M. Gahl, U. Frei, and P. Scholz. 1997. Inhibition of tumour necrosis factor production in endotoxin-stimulated human mononuclear leukocytes by the prostacyclin analogue iloprost: cellular mechanisms. *Cytokine 9:119.*
34. Yang, S. K., L. Eckmann, A. Panja, and M. F. Kagnoff. 1997. Differential and regulated expression of C-X-C, C-C, and C-chemokines by human colon epithelial cells. *Gastroenterology 113:1214.*
35. Albanesi, C., A. Cavani, and G. Girolomoni. 1999. IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effects with IFN-γ and TNF-α. *J. Immunol. 162:494.*
36. O'Bryan, J. P., R. A. Frye, P. C. Cogswell, A. Neubauer, B. Kitch, C. Prokop, R. Espinosa 3rd, M. M. Le Beau, H. S. Earp, and E. T. Liu. 1991. *axl,* a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. *Mol. Cell Biol. 11:5016.*
37. Ponte, P., S. Y. Ng, J. Engel, P. Gunning, and L. Kedes. 1984. Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human β-actin cDNA. *Nucleic Acids Res. 12:1687.*
38. Guo, X., H. Friess, H. U. Graber, M. Kashiwagi, A. Zimmermann, M. Korc, and M. W. Buchler. 1996. KAI1 expression is up-regulated in early pancreatic cancer and decreased in the presence of metastases. *Cancer Res. 56:4876.*
39. Friess, H., Y. Yamanaka, M. Buchler, H. G. Berger, M. S. Kobrin, R. L. Baldwin, and M. Korc. 1993. Enhanced expression of the type II transforming growth factor beta receptor in human pancreatic cancer cells without alteration of type III receptor expression. *Cancer Res. 53:2704.*

40. Friess, H., Y. Yamanaka, M. Buchler, M. Ebert, H. G. Beger, L. I. Gold, and M. Korc. 1993. Enhanced expression of transforming growth factor β isoforms in pancreatic cancer correlates with decreased survival. *Gastroenterology 105:1846.*
41. Lu, Z., H. Friess, H. U. Graber, X. Guo, M. Schilling, A. Zimmermann, M. Korc, and M. W. Buchler. 1997. Presence of two signaling TGF-β receptors in human pancreatic cancer correlates with advanced tumor stage. *Dig. Dis. Sci. 42:2054.*
42. Baker, N. E., G. Kucera, and A. Richmond. 1990. Nucleotide sequence of the human melanoma growth stimulatory activity (MGSA) gene. *Nucleic Acids Res. 18:6453.*
43. Rollins, B. J. 1997. Chemokines. *Blood 90:909.*
44. Schwarzenberger, P., R. La, V. A. Miller, P. Ye, W. Huang, A. Zieske, S. Nelson, G. J. Bagby, D. Stoltz, R. L. Mynatt, et al. 1998. IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for in vivo evaluation of cytokines. *J. Immunol. 161:6383.*
45. Laan, M., Z. H. Cui, H. Hoshino, J. Lotvall, M. Sjostrand, D. C. Gruenert, B. E. Skoogh, and A. Linden. 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J. Immunol. 162:2347.*
46. Hoshino, H., J. Lotvall, B. E. Skoogh, and A. Linden. 1999. Neutrophil recruitment by interleukin-17 into rat airways in vivo: role of tachykinins. *Am. J. Respir. Crit. Care Med. 159:1423.*
47. Ajuebor, M. N., A. M. Das, L. Virag, R. J. Flower, C. Szabo, and M. Perretti. 1999. Role of resident peritoneal macrophages and mast cells in chemokine production and neutrophil migration in acute inflammation: evidence for an inhibitory loop involving endogenous IL-10. *J. Immunol. 162:1685.*
48. Bozic, C. R., J. Kolakowski-LF, N. P. Gerard, R. C. Garcia, G. C. von Uexkull, M. J. Conklyn, R. Breslow, H. J. Showell, and C. Gerard. 1995. Expression and biologic characterization of the murine chemokine KC. *J. Immunol. 154:6048.*
49. Walley, K. R., N. W. Lukacs, T. J. Standiford, R. M. Strieter, and S. L. Kunkel. 1997. Elevated levels of macrophage inflammatory protein 2 in severe murine peritonitis increase neutrophil recruitment and mortality. *Infect. Immun. 65:3847.*
50. Frevert, C. W., A. Farone, H. Danaee, J. D. Paulauskis, and L. Kobzik. 1995. Functional characterization of rat chemokine macrophage inflammatory protein-2. *Inflammation 19:133.*
51. Mukaida, N., A. Harada, and K. Matsushima. 1998. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. *Cytokine Growth Factor Rev. 9:9.*
52. Betjes, M. G., C. W. Tuk, D. G. Struijk, R. T. Krediet, L. Arisz, M. Hart, and R. H. Beelen. 1993. Interleukin-8 production by human peritoneal mesothelial cells in response to tumor necrosis factor-α, interleukin-1, and medium conditioned by macrophages cocultured with *Staphylococcus epidermidis*. *J. Infect. Dis. 168:1202.*
53. Li, F. K., A. Davenport, R. L. Robson, P. Loetscher, R. Rothlein, J. D. Williams, and N. Topley. 1998. Leukocyte migration across human peritoneal mesothelial cells is dependent on directed chemokine secretion and ICAM-1 expression. *Kidney Int. 54:2170.*
54. Visser, C. E., J. J. Steenbergen, M. G. Betjes, S. Meijer, L. Arisz, E. C. Hoefsmit, R. T. Krediet, and R. H. Beelen. 1995. Interleukin-8 production by human mesothelial cells after direct stimulation with staphylococci. *Infect. Immun. 63:4206.*
55. Visser, C. E., J. Tekstra, J. J. Brouwer Steenbergen, C. W. Tuk, D. M. Boorsma, S. C. Sampat Sardjoepersad, S. Meijer, R. T. Krediet, and R. H. Beelen. 1998. Chemokines produced by mesothelial cells: huGRO-α, IP-10, MCP-1 and RANTES. *Clin. Exp. Immunol. 112:270.*
56. Attur, M. G., R. N. Patel, S. B. Abramson, and A. R. Amin. 1997. Interleukin-17 up-regulation of nitric oxide production in human osteoarthritis cartilage. *Arthritis Rheum. 40:1050.*
57. McColl, S. R., and I. Clark-Lewis. 1999. Inhibition of murine neutrophil recruitment in vivo by CXC chemokine receptor antagonists. *J. Immunol. 163:28.*
58. Chabaud, M., J. M. Durand, N. Buchs, F. Fossiez, G. Page, L. Frappart, and P. Miossec. 1999. Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. *Arthritis Rheum. 42:963.*
59. Kotake, S., N. Udagawa, N. Takahashi, K. Matsuzaki, K. Itoh, S. Ishiyama, S. Saito, K. Inoue, N. Kamatani, M. T. Gillespie, et al. 1999. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Invest. 103:1345.*
60. Antonysamy, M. A., W. C. Fanslow, F. Fu, W. Li, S. Qian, A. B. Troutt, and A. W. Thomson. 1999. Evidence for a role of IL-17 in organ allograft rejection: IL-17 promotes the functional differentiation of dendritic cell progenitors. *J. Immunol. 162:577.*