CMLS, Cell. Mol. Life Sci. 61 (2004) 567–579
1420-682X/04/050567-13
DOI 10.1007/s00018-003-3228-z

CMLS Cellular and Molecular Life Sciences

Review

Interleukin-17: a mediator of inflammatory responses

J. Witowski[a,b,*], K. Książek[a] and A. Jörres[b]

[a] Department of Pathophysiology, University Medical School, Święcickiego 6, Collegium Anatomicum, 60-781 Poznań (Poland), e-mail: jwitow@amp.edu.pl
[b] Department of Nephrology and Medical Intensive Care, Universitätsklinikum Charité, Berlin (Germany)

Received 13 June 2003; received after revision 27 August 2003; accepted 1 September 2003

Abstract. Interleukin-17 (IL-17) is a prototype member of a new cytokine family with six species identified to date. IL-17 is secreted mainly by activated $CD4^+$ and $CD8^+$ T lymphocytes, while its receptor is distributed ubiquitously. IL-17 has been classified as a proinflammatory cytokine because of its ability to induce the expression of many mediators of inflammation, most strikingly those that are involved in the proliferation, maturation and chemotaxis of neutrophils. Increased levels of IL-17 have been associated with several conditions, including airway inflammation, rheumatoid arthritis, intraperitoneal abscesses and adhesions, inflammatory bowel disease, allograft rejection, psoriasis, cancer and multiple sclerosis. This review provides an overview of IL-17 activities, concentrating on those that lead to neutrophil recruitment.

Key words. IL-17; T cells; inflammation; chemokines; granulopoiesis.

Introduction

Inflammation is a complex reaction of host defence mechanisms aiming at neutralization of an insult and restoring normal tissue structure and function. A key pathological event in an acute phase of many forms of inflammation is the recruitment of polymorphonuclear leukocytes in response to a perceived pathogen. This is especially important for innate immunity, which provides the first and fast line of defence for the host. The molecular mechanisms that orchestrate the influx of neutrophils to the site of inflammation are not entirely clear. It is now believed that cells traditionally viewed as those associated with adaptive immunity and tolerance, such as T lymphocytes, may also significantly contribute to and modulate the course of inflammatory reaction. Evidence has been accumulating to suggest that one of the molecules that may serve as a mediator of the T cell response to pathogens is interleukin-17 (IL-17) – an archetype member of a new cytokine family. Although identified only a decade ago, IL-17 has quickly captured the attention of the scientific community. As a result, it has become apparent that IL-17 represents a unique cytokine system that may be involved in a number of inflammatory diseases, but also transplant rejection and tumour growth [1–6].

* Corresponding author.

Discovery and expression of IL-17 cytokines

IL-17 (IL-17A)

IL–17, also referred to as IL-17A, was discovered in a search for T-cell-derived molecules with immune functions. It was cloned from a T cell hybridoma produced by fusion of a mouse cytotoxic T cell and rat T cell lymphoma [7]. It was originally believed to derive from mouse cells and to belong to a family of cytotoxic T-lymphocyte-associated antigens (CTLAs). In fact, however, it was derived from the rat lymphoma fusion partner [8, 9]. IL-17 was found to display striking homology to the pro-

tein encoded by the open reading frame 13 of *Herpesvirus saimiri* (HVS13) [10]. Sequence homology between human IL-17 and HVS13 is 75% at the nucleotide level and 72% at the amino acid level. *H. saimiri* is a naturally occurring benign pathogen of squirrel monkeys but induces fulminant lymphomas in many other New World primates. It is also capable of transforming human T cells to continuous growth in vitro [11]. Remarkable homology between IL-17 and HVS13 sequences has led to the hypothesis that during evolution, the virus captured a portion of the human IL-17 gene to gain a survival advantage during infection [10]. This strategy appears to be common among the herpesviruses, as exemplified by the presence of homologs of IL-10 in the Epstein-Barr virus and of IL-6 in the Kaposi sarcoma-associated virus [12–14]. Deletion of the IL-17 sequence from *Herpesvirus saimiri* does not appear to affect its ability to cause devastating lymphomas in cottontop tamarins [15]. However, the presence of IL-17 was found to increase the virulence of the vaccinia virus in mice, causing decreased natural killer (NK) cell activity and animal survival [16]. The human IL-17 gene was originally mapped on human chromosome 2q31 [7]; however, it has also been located in a sequence from a chromosome 6p12 clone [1, 17]. The gene encodes a 20–30 kDa protein of 155 amino acids [8, 18]. IL-17 polypeptide consists of a 19-amino-acid signal sequence followed by a 136-amino-acid mature segment. It contains at least one N-glycosylation site and six cysteine residues that form intermolecular bonds during dimerization [8]. Human IL-17 exhibits 63 and 58% amino acid identity compared with the mouse and rat sequences, respectively [9].

Sources of IL-17 appear to be rather restricted; expression of IL-17 has been detected mainly in activated $CD4^+$ and $CD8^+$ T lymphocytes (predominantly of the memory $CD45RO^+$ subset) [8, 18–21]. Later studies detected the presence of IL-17 messenger RNA (mRNA) transcripts also in neutrophils [22] and eosinophils [23]. In mice, the production of IL-17 has been reported to occur also in $TCR\alpha^+/\beta^+CD4^-CD8^-$ thymocytes [9]. Importantly, IL-17-producing T cells cannot be classified into either Th1 or Th2 subtypes, since the clones that release IL-17 have been reported to differ in their ability to produce IFN-γ, TNFα and IL-4 – the cytokines that typically identify Th1/Th2 classes [24–27].

Other IL-17 isoforms

In recent years several other proteins homologous to IL-17 have been identified and designated as IL-17B, IL-17C, IL-17D, IL-17E and IL-17F [17, 28–32]. These molecules have a molecular weight of 20–30 kDa and consist of 163–202 amino acids that bear 20–50% homology to IL-17, especially within the C-terminal region. They share four conserved cysteine residues that may participate in formation of intermolecular disulphide linkages.

In contrast to restricted expression of IL-17, the IL-17B mRNA can be detected in a wide range of tissues, including spinal cord, testis, stomach, small intestine, pancreas, prostate and ovary [28, 29]. IL-17C expression has been confined only to rare expression sequence tags (ESTs) in adult prostate and foetal kidney libraries [28]. IL-17D appears to be most homologous to IL-17B and is expressed widely in skeletal and heart muscle, brain, adipose tissue, lung and pancreas. The most abundant cellular source of IL-17E (classified also as IL-25 [33]) appears to be Th2-polarized T cells [33]. Mast cells have also been found to produce IL-17E/IL-25 upon immunoglobulin (Ig)E cross-linking [34]. At the tissue level transcripts of IL-17E/IL-25 were detected at very low levels in testis, kidney, pancreas and prostate [30, 35]. The expression pattern of IL-17F appears to be similar to that of IL-17 and includes only activated CD^+4 T cells and monocytes [32]. Analysis of the crystal structure of IL-17F has revealed that, surprisingly, IL-17 family members adopt a cysteine knot fold in a manner that is typical for the superfamily of cysteine knot proteins [17]. This family includes a number of growth factors (NGF, PDGF, TGF-β), which, however, have no significant sequence identity with IL-17. An IL-17F isoform, named ML-1, shares a significant amino acid sequence homology with IL-17F and is similarly expressed in activated T cells, but also in basophils and mast cells [36]. In addition, the tissue distribution of ML-1 differs from that of IL-17, since in contrast to IL-17, the most abundant presence of ML-1 transcripts was detected in liver, lung, placenta and ovary [36].

Detailed information on chromosome locations, amino acid sequence alignments, homology and evolutionary associations of IL-17 cytokines can be found in other review articles [1, 3].

Induction of IL-17

Under in vitro conditions T cells have been shown to release IL-17 in response to such nonspecific stimuli as ionomycin and phorbol 12-myristate 13-acetate [8], but also after stimulation with IL-15 [22, 37]. More recently it has transpired that the production of IL-17 and IL-17F can be triggered by IL-23 [38], a cytokine produced by dendritic cells [39]. Interestingly, IL-23 appears to stimulate IL-17 production mainly in memory, but not naïve T cells [38]. Also *Eschericia coli*-derived lipopolysaccharide has been found to induce the release of IL-17 from T cells, but it required the presence of macrophages – another antigen-presenting cell type [40]. Indeed, in vivo data have confirmed that in the course of pneumonia caused by Gram-negative bacteria, dendritic cells are stimulated to secrete

IL-23, which acts further to induce IL-17 in $CD4^+$ and $CD8^+$ T cells [21]. This effect appears to be mediated by bacterial lipopolysaccharide, which activates the Toll-like receptor 4 (TLR4) signalling pathway in antigen-presenting cells [21] (fig. 1 A). It has also been demonstrated that T cells can be driven to produce IL-17 when primed with cognate peptide and stimulated with microbial lipopeptides [27, 38]. In addition, peripheral blood mononuclear cells have been found to express IL-17 in response to the outer membrane protein from *Porphyromonas gingivalis* [41]. Increased expression of IL-17 has been detected in *Helicobacter pylori*-colonized gastric mucosa [42], synovial fluid of patients with *Borrelia burgdorferi*-induced arthritis [27], lung homogenates in experimental *Klebsiella pneumoniae* infection [43] and gingival tissue from patients with gingivitis [41]. The upregulation of IL-17E/IL-25 mRNA has been detected in the lung and gut of mice challenged with *Aspergillus fumigatus* and *Nippostrongylus brasiliensis* [44].

These observations indicate that IL-17 may act to serve as a mediator of infection-induced immune responses (see below). The precise mechanism by which cells generate IL-17 in response to stimulation has not been fully elucidated. Blocking experiments suggest that the process is calcineurin- and cyclic AMP (cAMP)-dependent [19, 20, 37].

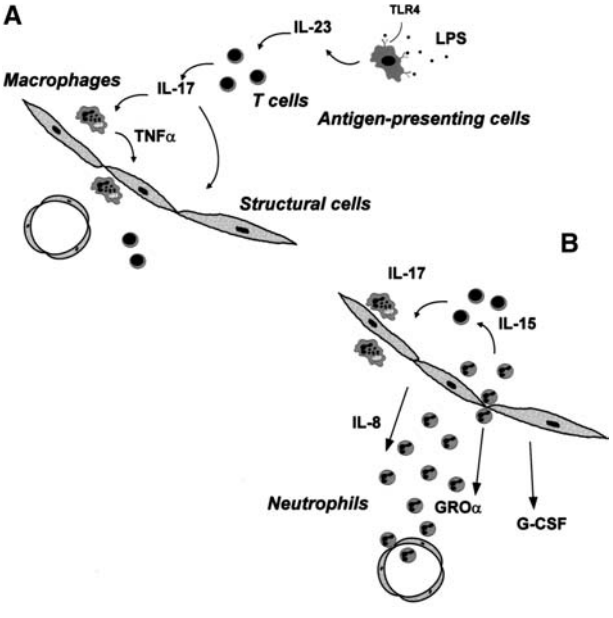

Figure 1. A suggested role of IL-17 in inflammatory response to bacterial infection. (*A*) Bacterial lipopolysaccharide stimulates antigen-presenting cells to produce IL-23, which then stimulates $CD4^+$ and $CD8^+$ T cells to release IL-17 [21]. IL-17 acts further on both tissue macrophages and structural cells. (*B*) In response to IL-17, cells generate neutrophil-specific CXC chemokines (IL-8, GROα) and granulopoietic cytokines (G-CSF, GM-CSF). By acting on macrophages, IL-17 stimulates the release of TNFα, which synergizes with IL-17 in its effects on structural cells. Accumulating neutrophils may produce IL-15 [22], which then may further stimulate T cells to release IL-17 [37].

IL-17 receptors

In contrast to relatively restricted expression of IL-17, receptors that bind IL-17 have been found to be ubiquitously expressed in all cell types and tissues examined [1, 3, 10, 45]. Surprisingly, it appears that the protein structure of IL-17R is unrelated to those of other cytokine receptor families. The human IL-17R gene has been mapped to chromosome 22 [45]. IL-17R complementary DNA (cDNA) encodes an extremely long type I transmembrane protein of 866 amino acids. The molecule includes an N-terminal signal peptide with a cleavage site after amino acid 27, followed by a 293-amino-acid extracellular domain, a 21-amino-acid transmembrane domain, and an unusually long cytoplasmic tail of 525 amino acids. The IL-17R chain contains at least seven N-linked glycosylation sites, and the molecular mass of nascent IL-17R protein is approximately 112 kDa. Binding studies have revealed that IL-17 binds to its receptor with relatively low affinity with K_a values of approximately $2 \times 10^7 - 2 \times 10^8$/M.

Recent studies show that new members of the IL-17 family bind preferentially to other forms of IL-17R. Newly characterized IL-17R homolog-1 (IL-17Rh1 or IL-17BR) was found to bind IL-17E [30] and to lesser extent also IL-17B [29]. IL-17Rh1 is a molecule of 48 kDa. It is made up of 426 amino acids, the sequence of which is 19% identical to that of IL-17R [29]. Compared with IL-17R, the smaller size of IL-17Rh1 appears to be predominantly related to the much shorter cytoplasmic domain. IL-17Rh1 mRNA expression is most pronounced in liver, kidney, pancreas, colon, small intestine and testis, but is absent from lymphoid organs and peripheral blood leukocytes [29]. Interestingly, IL-17Rh1 mRNA expression in rodents was found to be dramatically upregulated during intestinal inflammation [29].

Another form of IL-17R has been termed IL-17Rh2 or IL-17RL [46] and claimed to bind either IL-17F or IL-17B [1]. Its presence has been detected in a variety of tissues. Alternatively spliced IL-17Rh2 variants have been suggested to act as soluble decoy receptors antagonizing cytokine signalling [46]. In a very recent study Yang et al. have identified a new IL-17R-like protein (termed hSEF) [47]. It is expressed predominantly in endothelial and epithelial cells. Surprisingly, it does not appear to bind any IL-17 family members. Instead, it has been found to form complexes with a type 1 fibroblast growth factor receptor (FGFR1) and inhibit FGF signalling when overexpressed in human 293T kidney cells [47].

IL-17 signalling

IL-17 has been shown to induce expression of several cytokines known to contain nuclear factor kappa B (NF-κB)

binding sites in their promoters. Indeed, subsequent studies have demonstrated that IL-17 is capable of activating NF-κB transcription factors in many cell types, including fibroblasts [48], macrophages [49], chondrocytes [50, 51], intestinal epithelial cells [52, 53], and colonic and pancreatic myofibroblasts [54, 55]. In the absence of stimulation NF-κB is retained in cytoplasm in complexes with inhibitory κB (IκB) proteins. Upon stimulation, IκB proteins are phosphorylated by IκB kinases and rapidly degraded. It results in the release of NF-κB, which enters the nucleus and activates target genes. It has been demonstrated that activation of IκB kinases by IL-17 requires tumour necrosis factor (TNF) receptor-associated factor-6 (TRAF6) adapter protein as a signal transducer [48, 52]. In fibroblasts from TRAF6-deficient mice IL-17 fails to activate IκB kinases and consequently cannot induce NF-κB-dependent genes [48]. The activation of NFκB has also been demonstrated in response to IL-17D, IL-17E and IL-17F [31, 32, 56].

IL-17 signalling has been shown to use pathways regulated by three different classes of mitogen-activated protein kinases (MAPKs): extracellular signal-regulated kinases (ERK1 and ERK2), stress-induced c-Jun N-terminal kinases (JNK-1 and JNK-2), and p38 MAPK [50–52, 54, 55, 57–61]. ML-1-induced cytokine production has also been reported to be mediated by ERK (but not JNK and p38) [36]. It has been suggested that at least in renal epithelial cells, the upstream events leading to IL-17-induced MAPK may involve phosphorylation of src kinases [60]. In addition, it has been demonstrated that in the monocytic leukaemia cell line IL-17 induces a signalling pathway of Janus kinases (JAKs) and signal transducers and activators of transcription (STATs). It involves tyrosine phosphorylation of several members of the JAK/STAT family, including Tyk2, JAK1-3 and STAT1-4 [62].

Biological activity of IL-17

IL-17 and neutrophil migration

In vitro studies have demonstrated that by acting on a wide range of stromal cells, IL-17 induces a number of proinflammatory mediators (table 1). Interestingly, IL-17 appears to stimulate predominantly the production of cytokines that either specifically attract neutrophils to the site of inflammation (IL-8, GROα, GCP-2) or stimulate granulopoiesis in bone marrow (IL-6, G-CSF, GM-CSF). Moreover, IL-17 was found to induce IL-1β and TNFα in macrophages [49], and these cytokines can further synergize with IL-17 to amplify the synthesis of CXC neutrophil-specific chemokines and granulocyte/macrophage colony-stimulating factor (GM-CSF) [63–65]. In contrast, IL-17 appears to inhibit the TNFα- and interferon (IFN)-γ-stimulated production of RANTES, a chemokine which acts mainly on mononuclear leukocytes [66–68]. Since RANTES is a potent chemoattractant for lymphocytes, it has been speculated that the inhibition of RANTES secretion by lymphocyte-derived IL-17 may represent a regulatory mechanism limiting lymphocyte infiltration [68].

While IL-17 itself does not effect neutrophil chemotaxis in vitro [63], the supernatants from IL-17-treated fibroblasts, epithelial and endothelial cells stimulate neutrophil migration [63, 67]. This effect is largely related to IL-17-induced chemokines, since anti-chemokine antibodies can significantly reduce it. In vivo studies led to a similar conclusion since intratracheal or intraperitoneal adminis-

Table 1. Mediators induced by IL-17 in vitro.

Mediator	Cell type	References
CINC	intestinal epithelial cells	[52, 71]
Complement's C3	fibroblasts, renal proximal tubule epithelial cells	[71, 72]
IL-1	macrophages, chondrocytes	[49, 50]
IL-6	bronchial and renal epithelial cells, pancreatic periacinar and colonic myofibroblasts, chondrocytes, keratinocytes, fibroblasts, synoviocytes, cervical carcinoma and melanoma cell lines	[8, 18, 50, 54, 55, 59, 60, 71, 73–75]
IL-8	bronchial and renal epithelial cells, endothelial cells, pancreatic periacinar and colonic myofibroblasts, keratinocytes, fibroblasts, synoviocytes, cervical carcinoma and melanoma cell lines	[8, 18, 54, 58, 60, 63, 66, 71, 73, 75–77]
TNFα	macrophages	[49]
G-CSF	fibroblasts, synoviocytes, bronchial epithelial cells	[18, 76, 78]
GM-CSF	bronchial epithelial cells, venous endothelial cells	[65]
GROα	peritoneal mesothelial cells, bronchial fibroblasts and epithelial cells, synoviocytes, pancreatic periacinar myofibroblasts	[23, 58, 64, 76]
GCP-2	bronchial epithelial cells	[79]
ICAM-1	fibroblasts	[8]
MCP-1	renal epithelial cells, pancreatic periacinar and colonic myofibroblasts	[54, 60, 71, 77]
Nitric oxide	chondrocytes, astrocytes, endothelial cells	[50, 51, 80, 81]
PGE_2/COX_2	synoviocytes, chondrocytes, macrophages	[18, 50, 61]

tration of IL-17 [63, 64] or adenovirus-mediated IL-17 overexpression [43] resulted in a substantial induction of chemokines and neutrophil infiltration (fig. 1B). These observations were further confirmed in studies with genetically modified animals.

Although IL-17-deficient mice do not show any gross phenotypic abnormalities, they display suppressed hypersensitivity responses and decreased T-cell-dependent antibody production [69]. These effects are related to insufficient hapten-specific $CD4^+$ T cell activation in the sensitization phase. In addition, IL-17-deficiency results in reduced tissue chemokine expression and decreased neutrophil infiltration in the elicitation phase of immune responses. This observation is consistent with that made in IL-17 receptor-knockout mice [70]. These animals display reduced constitutive and stimulated expression of neutrophil-specific chemokines (MIP-2) and granulopoiesis-stimulating factors (G-CSF), which results in impaired neutrophil infiltration and increased mortality in response to infection.

IL-17 and granulopoiesis

In their seminal paper Fossiez et al. have demonstrated that IL-17 does not have a direct effect on human $CD34^+$ umbilical cord blood-derived stem cells in vitro [18]. However, when cultured together with IL-17-stimulated fibroblasts, these precursor cells proliferate and differentiate preferentially into neutrophils. It indicates that IL-17-treated fibroblasts secrete hematopoietic mediators that support the growth and differentiation of $CD34^+$ progenitor cells. Subsequent in vivo experiments by the group of Kolls et al. have demonstrated that adenovirus-mediated overexpression of IL-17 induces granulopoiesis in mice [82]. This effect can be partly attributed to the ability of IL-17 to stimulate the release of G-CSF and to induce the transmembrane form of stem cell factor (SCF) in bone marrow stromal cells. G-CSF and SCF synergize together, and both are required to effect optimal granulopoiesis in response to IL-17 [83]. Experiments with anti-G-CSF antibodies in Steel-Dickie mice, which do not express the transmembrane domain of SCF, indicate that there also exists a G-CSF/SCF-independent mechanism that contributes to IL-17-induced neutrophilia [83]. Further studies have demonstrated that IL-17 is also capable of mobilizing hemopoietic precursor cells and peripheral blood stem cells that have both short- and long-term repopulating ability and can rescue lethally irradiated mice [84]. Moreover, increased levels of both IL-17 and G-CSF have been detected in mice deficient in leukocyte adhesion molecules. These animals display significant neutrophilia that correlates with plasma levels of IL-17 and G-CSF and that can be reduced by blocking IL-17 and G-CSF [85, 86]. In this respect, it has been suggested that IL-17 and G-CSF form a regulatory axis important for neutrophil homeostasis and migration [85].

Effects of other IL-17 family members

Unlike IL-17, both IL-17B and IL-17C fail to induce IL-6 in fibroblasts but stimulate TNFα and IL-1β in the THP-1 monocytic leukaemia cell line [28]. IL-17D was found to induce the production of IL-6, IL-8 and GM-CSF in endothelial cells [31]. Surprisingly, however, it suppressed the proliferation of myeloid progenitors in colony formation assays [31]. This is in contrast to its ability to stimulate the production of GM-CSF, which supports progenitor cell maturation. IL-17F has been found to induce G-CSF and IL-8 in fibroblasts [17] and transforming growth factor (TGF)-β in endothelial cells [32]. Mice given an adenoviral IL-17F construct showed significant bronchoalveolar lavage (BAL) neutrophilia and increased pulmonary expression of cytokines associated with Th1 responses, including IL-6, IFN-γ, IFN-γ inducible protein-10 (IP-10) and monokine induced by IFN-γ (MIG). On the other hand, IL-17F was found to inhibit in vitro angiogenesis [32].

Biological activities of IL-17E/IL-25 seem to be quite different from those of other IL-17 cytokines. Although IL-17E/IL-25 was found to stimulate IL-8 synthesis in human kidney-derived cell lines in vitro [30], in vivo studies point rather to the involvement of IL-17E/IL-25 in Th2-mediated reactions. Administration or overexpression of IL-17E/IL-25 in mice resulted in marked eosinophilia, elevated IgE and increased tissue expression of IL-4, IL-5, IL-13 and eotaxin [35, 44, 87]. These changes were associated with chronic inflammation in multiple organs, including heart, lungs, lymph nodes and liver. Severe cholangitis and hepatitis gave rise to markedly elevated liver enzymes and jaundice.

Involvement of IL-17 in disease

Airway inflammation

Significant insight into the role of IL-17 in the airways has been gained by Kolls et al. from experiments, which employed a murine model of *Klebsiella pneumoniae* pulmonary infection [2]. In this setting the bacterial challenge resulted in the induction of IL-17 via a TLR4- and IL-23-dependent pathway [21, 43]. The biological relevance of IL-17 induction was clearly demonstrated by a homozygous deletion of the IL-17R gene in mice, which resulted in reduced chemokine levels and markedly diminished neutrophil recruitment into the lung during infection [70]. This led to impaired bacterial clearance and animal survival. In contrast, pretreatment with an adenovirus encoding IL-17 resulted in elevated chemokines, enhanced neutrophil influx and increased early survival

after challenge with bacteria [43]. These data point to an important role of IL-17 in pulmonary host defence mechanisms.

On the other hand, increasing evidence suggests that excessive IL-17 induction may contribute to airway inflammatory diseases such as chronic bronchitis, chronic obstructive pulmonary disease and severe exacerbation of asthma [1, 88–90]. All these conditions are characterized by the recruitment and activation of neutrophils in the airways. Indeed, increased levels of free, soluble IL-17 protein have been detected in BAL fluid obtained either from human airways in the course of inflammation caused by exposure to organic dust [91] or from mice exposed to intranasal endotoxin [40]. In both situations the elevation of IL-17 was associated with increased numbers of neutrophils in the airways. In mice, the degree of neutrophil infiltration could be reduced by the systemic blockade of IL-17 with a neutralizing antibody [40].

As indicated earlier, the mechanism by which IL-17 produces neutrophilia in the airways is most likely related to the induction of neutrophil-mobilizing cytokines. In vitro studies have demonstrated that bronchial epithelial cells stimulated by IL-17 release GROα, IL-8, GCP-2, G-CSF and GM-CSF [63, 65, 76, 79, 92]. In rodents, intratracheal administration or adenovirus-mediated overexpression of IL-17 in the lung also results in increased levels of cytokines such as MIP-2, IL-6 and G-CSF [40, 43, 63, 65]. Indeed, it has been found that neutrophil accumulation in the airways induced by either IL-17 or a combination of TNFα and IL-17 can be reduced by neutralization of MIP-2 and GM-CSF, respectively [63, 65]. Furthermore, it has been demonstrated that the neutrophil influx triggered by IL-17 may be modulated in vivo by endogenous tachykinins acting via NK-1 receptors [93]. Although IL-17 does not activate neutrophils in vitro, it may indirectly contribute to neutrophil activation in vivo, as evidenced by increased levels of myeloperoxidase and elastase in the airways of rats exposed to IL-17 [94].

The role of IL-17 in asthma remains to be fully elucidated. Immunocytochemical studies have detected an increased number of cells expressing IL-17 in sputum, BAL fluid [23] and bronchial biopsies [95] obtained from patients with asthma. In some asthmatic subjects challenged with an allergen, BAL cells have been found to express the IL-17F homolog, ML-1, rather than IL-17 [36]. On the other hand, the concentration of free IL-17 in asthmatic patients was found to be moderately elevated only in samples of BAL fluid [23], while the levels detected in serum and sputum, albeit seemingly higher, were not statistically different from those of healthy controls [96, 97]. In mice with experimental ovalbumin-induced allergic asthma, an increase in IL-17 mRNA expression in the lung has been observed upon acute antigen challenge [98]. The rise in IL-17 expression was accompanied by a significant neutrophil influx into the airways, which could be markedly reduced by pretreatment with anti-IL-17 antibodies [98]. Interestingly, decreased ovalbumin-induced airway hypersensitivity response and reduced pulmonary inflammation have also been observed in IL-17$^{-/-}$ × DO11.10 Tg mice which are deficient in IL-17 but bear ovalbumin-specific T cell receptor [69]. Decreased response of these animals to methacholine appears to be in line with a recent observation of increased IL-17 concentrations in the sputum of patients with bronchial hyperactivity [97]. Interestingly, IL-17 has also been found to stimulate the in vitro expression of mucin genes *MUC5B* and *MUC5AC* in tracheobronchial epithelial cells [99]. If occurring in vivo, this effect may contribute to mucus hypersecretion in asthma.

Although all these observations suggest IL-17 involvement in asthma, blocking IL-17 activity may not be as beneficial as expected. While neutralization of IL-17 in mice with experimental allergic asthma was found to effectively reduce the influx of neutrophils into the airways, it also caused a significant increase in IL-5 levels and exacerbation of eosinophilic lung inflammation [98]. It is also not clear how other IL-17 cytokines contribute to allergic reactions in the airways. As indicated earlier, mice treated with IL-17E/IL-25 show a Th2-biased immune response with increased pulmonary expression of IL-5, IL-13, eotaxin, marked eosinophilia and striking histological changes in the airways, including eosinophilic infiltrates, increased mucus production and epithelial cell hyperplasia [33, 44].

Peritoneal inflammation

Formation of intraabdominal adhesions and/or abscesses is a severe complication of peritoneal injury by surgery or infection. These events are commonly associated with intraperitoneal accumulation of neutrophils. In fact, however, the whole process may be controlled by CD4$^+$ T cells via an IL-17-dependent mechanism [100, 101]. Recent experimental studies in mice have revealed that the development of adhesions and abscesses following peritoneal injury or *Bacteroides fragilis* infection was associated with the accumulation of IL-17-producing T cells in the peritoneum. Administration of anti-IL-17 antibodies significantly reduced the degree of subsequent adhesion and abscess formation. In addition, the development of adhesions could be reduced by treatment with an antibody that blocks the receptor for CXC chemokines, CXC receptor type 2 (CXCR2) [101]. On the other hand, the intraperitoneal injection of recombinant IL-17 in mice caused a rapid increase in CXC chemokine levels (KC and MIP-2), followed by a selective influx of neutrophils [64]. Accordingly, anti-chemokine antibodies could suppress this IL-17-induced neutrophil infiltration. The main source of IL-17-induced chemokines appears to be the peritoneal mesothe-

lium: in vitro studies demonstrated a remarkable capacity of mesothelial cells to synthesize a CXC chemokine GROα in response to IL-17 [64]. These observations suggest that a link between T cells and peritoneal inflammation is mediated by IL-17 and neutrophil-specific chemokines. This view has gained further support from experiments in which animals depleted of T cells bearing $\alpha\beta$TCR or deficient in $CD4^+$ cells were found to produce less CXC chemokines and did not (or very rarely) develop abscesses or adhesions after peritoneal injury [100, 101].

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the destruction of articular cartilage and bone. Although T cells are present among the cells infiltrating the synovium, their precise role in the pathogenesis of disease has long been disputed. Recent studies indicate that the likely involvement of T cells is mediated to a significant extent by IL-17. First, elevated levels of IL-17 have been detected in the synovial fluid from patients with RA, but not with osteoarthritis [37, 102, 103]. Then, explants of the rheumatoid synovium were found to express and release IL-17 [104]. Immunostaining with anti-IL-17 antibodies identified T cells within the inflammatory infiltrates as a source of this cytokine, albeit the percentage of IL-17-producing T cells was estimated to be only ~1% [104]. Furthermore, it has been demonstrated that mononuclear cells isolated from the synovial fluid of RA patients produce IL-17 in response to IL-15, which can be found at high levels in the inflamed synovium [37]. Once released, IL-17 may act further on all other cell types in the rheumatoid joint [105]. In these actions IL-17 often synergizes with IL-1β and TNFα, which are expressed and upregulated at the protein and mRNA levels in the synovial tissue of RA patients [106–108]. In synoviocytes IL-17 has been found to stimulate or amplify IL-1β- and TNFα-induced production of cytokines, including IL-6, IL-8, growth-related oncogene α (GROα), leukaemia inhibitory factor (LIF) and MIP-3α [58, 109–111]. In the inflamed joint IL-17-induced CXC chemokines may attract neutrophils, while MIP-3α may be responsible for the recruitment of dendritic and T cells. On the other hand, IL-6 and LIF most probably contribute to cartilage destruction (reviewed in [112]). Other studies have demonstrated a reduced proteoglycan synthesis and an increased collagen breakdown in murine and bovine cartilage explants treated with IL-17 [113, 114]. The modulating role of IL-17 in the turnover of articular extracellular matrix may also be related to its ability to induce matrix metalloproteinases (MMPs) [103, 114–116].

Further compelling evidence of IL-17 involvement in the pathogenesis of RA came from animal studies. Intraarticular injection of IL-17 in mice resulted in joint inflammation and cartilage degradation [117, 118]. Similarly, adenovirus-mediated overexpression of IL-17 in the knee joint in the course of experimental collagen-induced arthritis in mice led to markedly aggravated joint destruction [119]. In contrast, neutralization of endogenous IL-17 significantly alleviated the extent of tissue damage in either collagen- or adjuvant-induced experimental arthritis [119, 120]. Recently, a beneficial effect of IL-17 blockade was also observed in a model of *Borellia burgdorferi*-induced arthritis [121]. In addition, IL-17 was found to mediate bone erosion in affected joints by inducing osteoclast formation [102]. Acting on stromal osteoblasts, IL-17 appears to stimulate COX-2-dependent prostaglandin E_2 (PGE_2) synthesis, which is required for the cells to express a membrane-associated osteoclast differentiation factor (ODF/RANKL). The ODF/RANKL protein conveys an essential signal for osteoclastogenesis sensed by specific ODF receptors (RANK) on osteoclast progenitors. Indeed, local adenovirus-mediated IL-17 overexpression in joints of mice with collagen-induced arthritis resulted in increased RANKL/RANK expression and osteoclastic bone destruction [122]. Very recently, markedly increased IL-17 expression has been documented in IL-1 receptor-antagonist-deficient ($IL\text{-}1Ra^{-/-}$) mice that spontaneously develop articular lesions resembling human rheumatoid arthritis [123]. When, however, $IL\text{-}1Ra^{-/-}$ animals are depleted also of IL-17 ($IL\text{-}17^{-/-} \times IL\text{-}1Ra^{-/-}$) they do not develop arthritis.

The specific activities of IL-17 cytokines in joint disease have recently been extensively reviewed [3].

Inflammatory bowel disease

The involvement of IL-17 in the pathogenesis of inflammatory bowel disease (IBD) has been suspected because of the presence of lymphocytic infiltrates in the inflamed colonic mucosa. Indeed, while IL-17 is absent from normal colorectal tissue, its expression has been detected in specimens from patients with active IBD [124, 125]. Double immunohistochemical staining localized the expression of IL-17 in $CD3^+$ T cells and $CD68^+$ monocytes [124]. Moreover, patients with active IBD have been found to have significantly elevated serum IL-17 concentrations compared with healthy individuals [124]. In vitro studies have demonstrated that IL-17 stimulates human colonic subepithelial myofibroblasts to produce both cytokines (IL-6, IL-8, MCP-1) [54] and a matrix metalloproteinase-3 [126]. Moreover, the IL-17-induced IL-6 synthesis can be further amplified by TNFα [54] and IL-4 [127]. In contrast, IL-17 downregulates TNFα-driven regulated upon activation normal T cell expressed and secreted (RANTES) synthesis in the same cells [68].

Demyelinating disorders

In multiple sclerosis increased expression of IL-17 has been detected both in brain lesions [128] and in mononuclear cells isolated from blood and cerebrospinal fluid [129]. Moreover, IL-17 has been found to modulate the course of experimental demyelinating inflammatory polyneuroparthy in rats [130]. Administration of IL-17 exacerbated the acute phase of neuritis, as shown by increased mononuclear cell infiltration of peripheral nerves, more extensive demyelination and elevated plasma TNFα levels. At later stages of disease, however, the animals receiving IL-17 made faster and complete recovery, compared with untreated rats.

Allograft rejection

IL-17 has been suggested to play a role in host versus graft reaction (HVGR). Increased IL-17 mRNA levels have been detected in rejected kidney transplants both in humans and in experimental animals [71, 131, 132]. Interestingly, IL-17 mRNA transcripts were consistently found in mononuclear cells of urinary sediment of patients with subclinical borderline rejection [132]. In vitro studies have shown that in human proximal tubular epithelial cells IL-17 stimulates the production of IL-6, IL-8 and MCP-1 [71], and synergizes with another T-cell-derived mediator, CD40L, to induce RANTES [133]. Therefore in the clinical situation, IL-17-induced chemokines may be responsible for the influx of leukocytes into the graft and subsequent tissue destruction. The blockade of IL-17 activity with an IL-17R domain fused to a Fc IgG fragment was found to inhibit T cell proliferation in vitro and to prolong acute survival of vascularized and nonvascularized heart allografts [134, 135]. However, neutralization of IL-17 did not prevent chronic rejection of aortic transplants in mice [136]. In addition, the observations made in IL-17-deficient mice do not suggest the IL-17 involvement in acute GVHR [69].

Inflammatory diseases of skin and cornea

IL-17 expression could not be detected in normal skin, but was found in skin lesions in allergic contact dermatitis and psoriasis [66, 73]. It was also observed in corneas of patients with fulminant herpetic stromal keratitis [67]. Interestingly, the expression of IL-17 was detected in T cell clones derived from these skin and corneal lesions. In addition, in keratinocytes IL-17 has been shown to induce IL-6, IL-8, GROα, GM-CSF and ICAM-1 by acting either directly or in combination with IFN-γ, IL-4 and TNFα [26, 66, 73]. In human corneal fibroblasts, the combination of IL-17 together with TNFα synergistically increased the production of IL-6, IL-8, MIP-1α and MIP-3α [67]. Interestingly, as in colonic myofibroblasts, IL-17 was found to inhibit the TNFα-stimulated release of RANTES both from keratinocytes [66] and corneal fibroblasts [67]. The involvement of IL-17 in allergic contact dermatitis has gained further support by an observation of markedly reduced contact hypersensitivity responses in IL-17-knockout mice [69].

Tumour growth

Several studies addressed the issue of IL-17 function in cancer. However, the data obtained so far appear to be conflicting. There are reports showing that IL-17 may support tumour growth, probably by stimulating angiogenesis [137, 138]. In contrast, other studies suggest that IL-17 may promote T-cell-mediated tumour rejection [139–141]. For an extensive review of IL-17 activities in cancer, the reader is directed elsewhere [3].

Concluding remarks

IL-17 appears to be an important mediator of inflammation, especially in neutrophil-dominated responses to bacterial challenge. This connection is intriguing given that expression of IL-17 is restricted to memory T cells, which are associated with an adaptive immune response, while neutrophils are viewed primarily as mediators of innate immunity. It has been hypothesized that by secreting IL-17, which subsequently induces chemokines and granulopoietic factors, memory T cells may enhance faster and more effective recruitment of neutrophils [142]. In this respect IL-17 may serve as a modulator of early immune responses to pathogens, and as such may be an important element of host defence. On the other hand, the overproduction of IL-17 may aggravate inflammatory reactions and contribute to tissue injury. In such situations IL-17 may be viewed as a potential target for therapeutic intervention, and this approach is now intensively being explored by the pharmaceutical industry (reviewed in [1]). Of other IL-17 family members, IL-17E/IL-25 appears to be of particular interest because of its possible involvement in Th2-mediated reactions.

Acknowledgement. J. W. and K. K. were supported by a grant from the Polish Scientific Research Committee (4 P05A 121 19).

1 Dumont F. J. (2003) IL-17 cytokine/receptor families: emerging targets for the modulation of inflammatory responses. Expert Opin. Ther. Patents **13:** 287–303
2 Kolls J. K., Kanaly S. T. and Ramsay A. J. (2003) Interleukin-17: an emerging role in lung inflammation. Am. J. Respir. Cell Mol. Biol. **28:** 9–11
3 Moseley T. A., Haudenschild D. R., Rose L. and Reddi A. H. (2003) Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev. **14:** 155–174
4 Schwarzenberger P. and Kolls J. K. (2002) Interleukin 17: an example for gene therapy as a tool to study cytokine mediated regulation of hematopoiesis. J. Cell Biochem. Suppl. **38:** 88– 95

5 Linden A. and Adachi M. (2002) Neutrophilic airway inflammation and IL-17. Allergy **57:** 769–775
6 Spriggs M. K. (1997) Interleukin-17 and its receptor. J. Clin. Immunol. **17:** 366–369
7 Rouvier E., Luciani M. F., Mattei M. G., Denizot F. and Golstein P. (1993) CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a *Herpesvirus saimiri* gene. J. Immunol. **150:** 5445–5456
8 Yao Z., Painter S. L., Fanslow W. C., Ulrich D., Macduff B. M., Spriggs M. K. et al. (1995) Human IL-17: a novel cytokine derived from T cells. J. Immunol. **155:** 5483–5486
9 Kennedy J., Rossi D. L., Zurawski S. M., Vega F. Jr, Kastelein R. A., Wagner J. L. et al. (1996) Mouse IL-17: a cytokine preferentially expressed by alpha beta TCR^+ $CD4^-CD8^-$ T cells. J. Interferon Cytokine Res. **16:** 611–617
10 Yao Z., Fanslow W. C., Seldin M. F., Rousseau A. M., Painter S. L., Comeau M. R. et al. (1995) *Herpesvirus saimiri* encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity **3:** 811–821
11 Biesinger B., Mueller-Fleckenstein I., Simmer B., Lang G., Wittmann S., Platzer E. et al. (1992) Stable growth transformation of human T-lymphocytes by *Herpevirus saimiri*. Proc. Natl. Acad. Sci. USA **89:** 3116–3319
12 Moore P. S., Boshoff C., Weiss R. A. and Chang Y. (1996) Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. Science **274:** 1739–1744
13 Moore K. W., Vieira P., Fiorentino D. F., Trounstine M. L., Khan T. A. and Mosmann T. R. (1990) Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. Science **248:** 1230–1234
14 Alcami A. (2003) Viral mimicry of cytokines, chemokines and their receptors. Nat. Rev. Immunol. **3:** 36–50
15 Knappe A., Hiller C., Niphuis H., Fossiez F., Thurau M., Wittmann S. et al. (1998) The interleukin-17 gene of *Herpesvirus saimiri*. J. Virol. **72:** 5797–5801
16 Patera A. C., Pesnicak L., Bertin J. and Cohen J. I. (2002) Interleukin 17 modulates the immune response to vaccinia virus infection. Virology **299:** 56–63
17 Hymowitz S. G., Filvaroff E. H., Yin J. P., Lee J., Cai L., Risser P. et al. (2001) IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F and implications for receptor binding. EMBO J. **20:** 5332–5341
18 Fossiez F., Djossou O., Chomarat P., Flores R. L., Ait Y. S., Maat C. et al. (1996) T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J. Exp. Med. **183:** 2593–2603
19 Shin H. C., Benbernou N., Esnault S. and Guenounou M. (1999) Expression of IL-17 in human memory $CD45RO^+$ T lymphocytes and its regulation by protein kinase A pathway. Cytokine **11:** 257–266
20 Shin H. C., Benbernou N., Fekkar H., Esnault S. and Guenounou M. (1998) Regulation of IL-17, IFN-gamma and IL-10 in human $CD8(^+)$ T cells by cyclic AMP-dependent signal transduction pathway. Cytokine **10:** 841–850
21 Happel K. I., Zheng M., Young E., Quinton L. J., Lockhart E., Ramsay A. J. et al. (2003) Roles of Toll-like receptor 4 and IL-23 in IL-17 expression in response to *Klebsiella pneumoniae* infection. J. Immunol. **170:** 4432–4436
22 Ferretti S., Bonneau O., Dubois G. R., Jones C. E. and Trifilieff A. (2003) IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger. J. Immunol. **170:** 2106–2112
23 Molet S., Hamid Q., Davoine F., Nutku E., Taha R., Page N. et al. (2001) IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. J. Allergy Clin. Immunol. **108:** 430–438
24 Aarvak T., Chabaud M., Miossec P. and Natvig J. B. (1999) IL-17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. J. Immunol. **162:** 1246–1251
25 Aarvak T., Chabaud M., Kallberg E., Miossec P. and Natvig J. B. (1999) Change in the Th1/Th2 phenotype of memory T-cell clones from rheumatoid arthritis synovium. Scand. J. Immunol. **50:** 1–9
26 Albanesi C., Scarponi C., Cavani A., Federici M., Nasorri F. and Girolomoni G. (2000) Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon-gamma- and interleukin-4-induced activation of human keratinocytes. J. Invest. Dermatol. **115:** 81–87
27 Infante-Duarte C., Horton H. F., Byrne M. C. and Kamradt T. (2000) Microbial lipopeptides induce the production of IL-17 in Th cells. J. Immunol. **165:** 6107–6115
28 Li H., Chen J., Huang A., Stinson J., Heldens S., Foster J. et al. (2000) Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. Proc. Natl. Acad. Sci. USA **97:** 773–778
29 Shi Y., Ullrich S. J., Zhang J., Connolly K., Grzegorzewski K. J., Barber M. C. et al. (2000) A novel cytokine receptor-ligand pair. Identification, molecular characterization and in vivo immunomodulatory activity. J. Biol. Chem. **275:** 19167–19176
30 Lee J., Ho W. H., Maruoka M., Corpuz R. T., Baldwin D. T., Foster J. S. et al. (2001) IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. J. Biol. Chem. **276:** 1660–1664
31 Starnes T., Broxmeyer H. E., Robertson M. J. and Hromas R. (2002) IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. J. Immunol. **169:** 642–646
32 Starnes T., Robertson M. J., Sledge G., Kelich S., Nakshatri H., Broxmeyer H. E. et al. (2001) IL-17F, a novel cytokine selectively expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. J. Immunol. **167:** 4137–4140
33 Fort M. M., Cheung J., Yen D., Li J., Zurawski S. M., Lo S. et al. (2001) IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity **15:** 985–995
34 Ikeda K., Nakajima H., Suzuki K., Kagami S., Hirose K., Suto A. et al. (2003) Mast cells produce interleukin-25 upon Fc epsilon RI-mediated activation. Blood **101:** 3594–3596
35 Kim M. R., Manoukian R., Yeh R., Silbiger S. M., Danilenko D. M., Scully S. et al. (2002) Transgenic overexpression of human IL-17E results in eosinophilia, B-lymphocyte hyperplasia and altered antibody production. Blood **100:** 2330–2340
36 Kawaguchi M., Onuchic L. F., Li X. D., Essayan D. M., Schroeder J., Xiao H. Q. et al. (2001) Identification of a novel cytokine, ML-1, and its expression in subjects with asthma. J. Immunol. **167:** 4430–4435
37 Ziolkowska M., Koc A., Luszczykiewicz G., Ksiezopolska-Pietrzak K., Klimczak E., Chwalinska-Sadowska H. et al. (2000) High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. J. Immunol. **164:** 2832–2838
38 Aggarwal S., Ghilardi N., Xie M. H., de Sauvage F. J. and Gurney A. L. (2003) Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. J. Biol. Chem. **278:** 1910–1914
39 Oppmann B., Lesley R., Blom B., Timans J. C., Xu Y., Hunte B. et al. (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity **13:** 715–725
40 Miyamoto M., Prause O., Sjostrand M., Laan M., Lotvall J. and Linden A. (2003) Endogenous IL-17 as a mediator of neutrophil recruitment caused by endotoxin exposure in mouse airways. J. Immunol. **170:** 4665–4672
41 Oda T., Yoshie H. and Yamazaki K. (2003) Porphyromonas gingivalis antigen preferentially stimulates T cells to express

IL-17 but not receptor activator of NF-kappaB ligand in vitro. Oral Microbiol. Immunol. **18:** 30–36
42 Luzza F., Parrello T., Monteleone G., Sebkova L., Romano M., Zarrilli R. et al. (2000) Up-regulation of IL-17 is associated with bioactive IL-8 expression in *Helicobacter pylori*-infected human gastric mucosa. J. Immunol. **165:** 5332–5337
43 Ye P., Garvey P. B., Zhang P., Nelson S., Bagby G., Summer W. R. et al. (2001) Interleukin-17 and lung host defense against *Klebsiella pneumoniae* infection. Am. J. Respir. Cell Mol. Biol. **25:** 335–340
44 Hurst S. D., Muchamuel T., Gorman D. M., Gilbert J. M., Clifford T., Kwan S. et al. (2002) New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. J. Immunol. **169:** 443–453
45 Yao Z., Spriggs M. K., Derry J. M., Strockbine L., Park L. S., VandenBos T. et al. (1997) Molecular characterization of the human interleukin (IL)-17 receptor. Cytokine **9:** 794–800
46 Haudenschild D. R., Moseley T. A., Rose L. M. and Reddi A. H. (2001) Soluble and transmembrane isoforms of novel interleukin-17 receptor-like protein by RNA splicing, and expression in prostate cancer. J. Biol. Chem. **277:** 4309–4316
47 Yang R. B., Ng C. K., Wasserman S. M., Komuves L. G., Gerritsen M. E. and Topper J. N. (2003) A novel IL-17 receptor-like protein identified in human umbilical vein endothelial cells antagonizes basic fibroblast growth factor-induced signaling. J. Biol. Chem. **278:** 33232–33238
48 Schwandner R., Yamaguchi K. and Cao Z. (2000) Requirement of tumor necrosis factor receptor-associated factor (TRAF)6 in interleukin 17 signal transduction. J. Exp. Med. **191:** 1233–1240
49 Jovanovic D. V., Di Battista J. A., Martel P. J., Jolicoeur F. C., He Y., Zhang M. et al. (1998) IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J. Immunol. **160:** 3513–3521
50 Shalom-Barak T., Quach J. and Lotz M. (1998) Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NF-kappaB. J. Biol. Chem. **273:** 27467–27473
51 Martel-Pelletier J., Mineau F., Jovanovic D., Di Battista J. A. and Pelletier J. P. (1999) Mitogen-activated protein kinase and nuclear factor kappaB together regulate interleukin-17-induced nitric oxide production in human osteoarthritic chondrocytes: possible role of transactivating factor mitogen-activated protein kinase-activated proten kinase (MAPKAPK). Arthritis Rheum. **42:** 2399–2409
52 Awane M., Andres P. G., Li D. J. and Reinecker H. C. (1999) NF-kappa B-inducing kinase is a common mediator of IL-17-, TNF-alpha- and IL-1 beta-induced chemokine promoter activation in intestinal epithelial cells. J. Immunol. **162:** 5337–5344
53 Andoh A., Takaya H., Makino J., Sato H., Bamba S., Araki Y. et al. (2001) Cooperation of interleukin-17 and interferon-gamma on chemokine secretion in human fetal intestinal epithelial cells. Clin. Exp. Immunol. **125:** 56–63
54 Hata K., Andoh A., Shimada M., Fujino S., Bamba S., Araki Y. et al. (2002) IL-17 stimulates inflammatory responses via NF-kappaB and MAP kinase pathways in human colonic myofibroblasts. Am. J. Physiol. Gastrointest. Liver Physiol. **282:** G1035–G1044
55 Shimada M., Andoh A., Hata K., Tasaki K., Araki Y., Fujiyama Y. et al. (2002) IL-6 secretion by human pancreatic periacinar myofibroblasts in response to inflammatory mediators. J. Immunol. **168:** 861–868
56 Lee J., Ho W. H., Maruoka M., Corpuz R. T., Baldwin D. T., Foster J. S. et al. (2001) IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. J. Biol. Chem. **276:** 1660–1664
57 Andoh A., Shimada M., Bamba S., Okuno T., Araki Y., Fujiyama Y. et al. (2002) Extracellular signal-regulated kinases 1 and 2 participate in interleukin-17 plus tumor necrosis factor-alpha-induced stabilization of interleukin-6 mRNA in human pancreatic myofibroblasts. Biochim. Biophys. Acta **1591:** 69–74
58 Kehlen A., Thiele K., Riemann D. and Langner J. (2002) Expression, modulation and signalling of IL-17 receptor in fibroblast-like synoviocytes of patients with rheumatoid arthritis. Clin. Exp. Immunol. **127:** 539–546
59 Laan M., Lotvall J., Chung K. F. and Linden A. (2001) IL-17-induced cytokine release in human bronchial epithelial cells in vitro: role of mitogen-activated protein (MAP) kinases. Br. J. Pharmacol. **133:** 200–206
60 Hsieh H. G., Loong C. C. and Lin C. Y. (2002) Interleukin-17 induces src/MAPK cascades activation in human renal epithelial cells. Cytokine **19:** 159–174
61 Faour W. H., Mancini A., He Q. W. and Di Battista J. A. (2003) T-cell derived interleukin-17 regulates the level and stability of cyclooxygenase -2 (COX-2) mRNA through restricted activation of the p38 mitogen-activated protein kinase casade. Role of distal sequences in the 3′-untranslated region (UTR) of COX-2 mRNA. J. Biol. Chem. **278:** 26897–26907
62 Subramaniam S. V., Cooper R. S. and Adunyah S. E. (1999) Evidence for the involvement of JAK/STAT pathway in the signaling mechanism of interleukin-17. Biochem. Biophys. Res. Commun. **262:** 14–19
63 Laan M., Cui Z. H., Hoshino H., Lotvall J., Sjostrand M., Gruenert D. C. et al. (1999) Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. J. Immunol. **162:** 2347–2352
64 Witowski J., Pawlaczyk K., Breborowicz A., Scheuren A., Kuzlan-Pawlaczyk M., Wisniewska J. et al. (2000) IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. J. Immunol. **165:** 5814–5821
65 Laan M., Prause O., Miyamoto M., Sjostrand M., Hytonen A. M., Kaneko T. et al. (2003) A role of GM-CSF in the accumulation of neutrophils in the airways caused by IL-17 and TNF-alpha. Eur. Respir. J. **21:** 387–393
66 Albanesi C., Cavani A. and Girolomoni G. (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-gamma and TNF-alpha. J. Immunol. **162:** 494–502
67 Maertzdorf J., Osterhaus A. D. and Verjans G. M. (2002) IL-17 expression in human herpetic stromal keratitis: modulatory effects on chemokine production by corneal fibroblasts. J. Immunol. **169:** 5897–5903
68 Andoh A., Fujino S., Bamba S., Araki Y., Okuno T., Bamba T. et al. (2002) IL-17 selectively down-regulates TNF-alpha-induced RANTES gene expression in human colonic subepithelial myofibroblasts. J. Immunol. **169:** 1683–1687
69 Nakae S., Komiyama Y., Nambu A., Sudo K., Iwase M., Homma I. et al. (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity **17:** 375–387
70 Ye P., Rodriguez F. H., Kanaly S., Stocking K. L., Schurr J., Schwarzenberger P. et al. (2001) Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment and host defense. J. Exp. Med. **194:** 519–527
71 van Kooten C., Boonstra J. G., Paape M. E., Fossiez F., Banchereau J., Lebecque S. et al. (1998) Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. J. Am. Soc. Nephrol. **9:** 1526–1534
72 Katz Y., Nadiv O., Rapoport M. J. and Loos M. (2000) IL-17 regulates gene expression and protein synthesis of the com-

plement system, C3 and factor B, in skin fibroblasts. Clin. Exp. Immunol. **120:** 22–29
73 Teunissen M. B., Koomen C. W., de Waal M. R., Wierenga E. A. and Bos J. D. (1998) Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. J. Invest. Dermatol. **111:** 645–649
74 Katz Y., Nadiv O. and Beer Y. (2001) Interleukin-17 enhances tumor necrosis factor alpha-induced synthesis of interleukins 1,6 and 8 in skin and synovial fibroblasts: a possible role as a 'fine-tuning cytokine' in inflammation processes. Arthritis Rheum. **44:** 2176–2184
75 Tartour E., Fossiez F., Joyeux I., Galinha A., Gey A., Claret E. et al. (1999) Interleukin 17, a T-cell-derived cytokine, promotes tumorigenicity of human cervical tumors in nude mice. Cancer Res. **59:** 3698–3704
76 Jones C. E. and Chan K. (2002) Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha and granulocyte-colony-stimulating factor by human airway epithelial cells. Am. J. Respir. Cell Mol. Biol. **26:** 748–753
77 Takaya H., Andoh A., Makino J., Shimada M., Tasaki K., Araki Y. et al. (2002) Interleukin-17 stimulates chemokine (interleukin-8 and monocyte chemoattractant protein-1) secretion in human pancreatic periacinar myofibroblasts. Scand. J. Gastroenterol. **37:** 239–245
78 Cai X. Y., Gommoll-CP J., Justice L., Narula S. K. and Fine J. S. (1998) Regulation of granulocyte colony-stimulating factor gene expression by interleukin-17. Immunol. Lett. **62:** 51–58
79 Prause O., Laan M., Lotvall J. and Linden A. (2003) Pharmacological modulation of interleukin-17-induced GCP-2-, GRO-alpha- and interleukin-8 release in human bronchial epithelial cells. Eur. J. Pharmacol. **462:** 193–198
80 Trajkovic V., Stosic-Grujicic S., Samardzic T., Markovic M., Miljkovic D., Ramic Z. et al. (2001) Interleukin-17 stimulates inducible nitric oxide synthase activation in rodent astrocytes. J. Neuroimmunol. **119:** 183–191
81 Miljkovic D., Cvetkovic I., Vuckovic O., Stosic-Grujicic S., Mostarica S. M. and Trajkovic V. (2003) The role of interleukin-17 in inducible nitric oxide synthase-mediated nitric oxide production in endothelial cells. Cell. Mol. Life Sci. **60:** 518–525
82 Schwarzenberger P., La Russa V., Miller A., Ye P., Huang W., Zieske A. 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:** 6383–6389
83 Schwarzenberger P., Huang W., Ye P., Oliver P., Manuel M., Zhang Z. et al. (2000) Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis. J. Immunol. **164:** 4783–4789
84 Schwarzenberger P., Huang W., Oliver P., Byrne P., La Russa V., Zhang Z. et al. (2001) IL-17 mobilizes peripheral blood stem cells with short- and long-term repopulating ability in mice. J. Immunol. **167:** 2081–2086
85 Forlow S. B., Schurr J. R., Kolls J. K., Bagby G. J., Schwarzenberger P. O. and Ley K. (2001) Increased granulopoiesis through interleukin-17 and granulocyte colony-stimulating factor in leukocyte adhesion molecule-deficient mice. Blood **98:** 3309–3314
86 Miyamoto M., Emoto M., Emoto Y., Brinkmann V., Yoshizawa I., Seiler P. et al. (2003) Neutrophilia in LFA-1-deficient mice confers resistance to listeriosis: possible contribution of granulocyte-colony-stimulating factor and IL-17. J. Immunol. **170:** 5228–5234
87 Pan G., French D., Mao W., Maruoka M., Risser P., Lee J. et al. (2001) Forced expression of murine IL-17E induces growth retardation, jaundice, a Th2-biased response and multiorgan inflammation in mice. J. Immunol. **167:** 6559–6567
88 Laan M. and Linden (2002) IL-17 as a potential target for modulating airway neutrophilia. Curr. Pharm. Des. **8:** 1855–1861
89 Linden A. (2001) Role of interleukin-17 and the neutrophil in asthma. Int. Arch. Allergy Immunol. **126:** 179–184
90 Linden A., Hoshino H. and Laan M. (2000) Airway neutrophils and interleukin-17. Eur. Respir. J. **15:** 973–977
91 Laan M., Palmberg L., Larsson K. and Linden A. (2002) Free, soluble interleukin-17 protein during severe inflammation in human airways. Eur. Respir. J. **19:** 534–537
92 Kawaguchi M., Kokubu F., Kuga H., Matsukura S., Hoshino H., Ieki K. et al. (2001) Modulation of bronchial epithelial cells by IL-17. J. Allergy Clin. Immunol. **108:** 804–809
93 Hoshino H., Lotvall J., Skoogh B. E. and Linden A. (1999) Neutrophil recruitment by interleukin-17 into rat airways in vivo. Role of tachykinins. Am. J. Respir. Crit. Care Med. **159:** 1423–1428
94 Hoshino H., Laan M., Sjostrand M., Lotvall J., Skoogh B. E. and Linden A. (2000) Increased elastase and myeloperoxidase activity associated with neutrophil recruitment by IL-17 in airways in vivo. J. Allergy Clin. Immunol. **105:** 143–149
95 Chakir J., Shannon J., Molet S., Fukakusa M., Elias J., Laviolette M. et al. (2003) Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. J. Allergy Clin. Immunol. **111:** 1293–1298
96 Wong C. K., Ho C. Y., Ko F. W., Chan C. H., Ho A. S., Hui D. S. et al. (2001) Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN-gamma, IL-4, IL-10 and IL-13) in patients with allergic asthma. Clin. Exp. Immunol. **125:** 177–183
97 Barczyk A., Pierzchala W. and Sozanska E. (2003) Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. Respir. Med. **97:** 726–733
98 Hellings P. W., Kasran A., Liu Z., Vandekerckhove P., Wuyts A., Overbergh L. et al. (2003) Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. Am. J. Respir. Cell Mol. Biol. **28:** 42–50
99 Chen Y., Thai P., Zhao Y. H., Ho Y. S., DeSouza M. M. and Wu R. (2003) Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. J. Biol. Chem. **278:** 17036–17043
100 Chung D. R., Kasper D. L., Panzo R. J., Chtinis T., Grusby M. J., Sayegh M. H. et al. (2003) $CD4^+$ T cells mediate abscess formation in intra-abdominal sepsis by an IL-17-dependent mechanism. J. Immunol. **170:** 1958–1963
101 Chung D. R., Chitnis T., Panzo R. J., Kasper D. L., Sayegh M. H. and Tzianabos A. O. (2002) $CD4^+$ T cells regulate surgical and postinfectious adhesion formation. J. Exp. Med. **195:** 1471–1478
102 Kotake S., Udagawa N., Takahashi N., Matsuzaki K., Itoh K., Ishiyama S. et al. (1999) IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J. Clin. Invest. **103:** 1345–1352
103 Jovanovic D. V., Martel-Pelletier J., Di Battista J. A., Mineau F., Jolicoeur F. C., Benderdour M. et al. (2000) Stimulation of 92-kd gelatinase (matrix metalloproteinase 9) production by interleukin-17 in human monocyte/macrophages: a possible role in rheumatoid arthritis. Arthritis Rheum. **43:** 1134–1144
104 Chabaud M., Durand J. M., Buchs N., Fossiez F., Page G., Frappart L. et al. (1999) Human interleukin-17: A T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. Arthritis Rheum. **42:** 963–970
105 Miossec P. (2003) Interleukin-17 in rheumatoid arthritis: if T cells were to contribute to inflammation and destruction through synergy. Arthritis Rheum. **48:** 594–601
106 Buchan G., Barrett K., Turner M., Chantry D., Maini R. N. and Feldmann M. (1988) Interleukin-1 and tumour necrosis factor

mRNA expression in rheumatoid arthritis: prolonged production of IL-1 alpha. Clin. Exp. Immunol. **73:** 449–455
107 Saxne T., Palladino M. A. Jr, Heinegard D., Talal N. and Wollheim F. A. (1988) Detection of tumor necrosis factor alpha but not tumor necrosis factor beta in rheumatoid arthritis synovial fluid and serum. Arthritis Rheum. **31:** 1041–1045
108 Hopkins S. J., Humphreys M. and Jayson M. I. (1988) Cytokines in synovial fluid. I. The presence of biologically active and immunoreactive IL-1. Clin. Exp. Immunol. **72:** 422–427
109 Kehlen A., Pachnio A., Thiele K. and Langner J. (2003) Gene expression induced by interleukin-17 in fibroblast-like synoviocytes of patients with rheumatoid arthritis: upregulation of hyaluronan-binding protein TSG-6. Arthritis Res. Ther. **5:** R186–R192
110 Chabaud M., Page G. and Miossec P. (2001) Enhancing effect of IL-1, IL-17 and TNF-alpha on macrophage inflammatory protein-3alpha production in rheumatoid arthritis: regulation by soluble receptors and Th2 cytokines. J. Immunol. **167:** 6015–6020
111 Chabaud M., Fossiez F., Taupin J. L. and Miossec P. (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:** 409–414
112 Wong P. K., Campbell I. K., Egan P. J., Ernst M. and Wicks I. P. (2003) The role of the interleukin-6 family of cytokines in inflammatory arthritis and bone turnover. Arthritis Rheum. **48:** 1177–1189
113 Lubberts E., Joosten L. A., van de Loo F. A., van den Gersselaar L. A. and van Den Berg W. B. (2000) Reduction of interleukin-17-induced inhibition of chondrocyte proteoglycan synthesis in intact murine articular cartilage by interleukin-4. Arthritis Rheum. **43:** 1300–1306
114 Koshy P. J., Henderson N., Logan C., Life P. F., Cawston T. E. and Rowan A. D. (2002) Interleukin 17 induces cartilage collagen breakdown: novel synergistic effects in combination with proinflammatory cytokines. Ann. Rheum. Dis. **61:** 704–713
115 Chabaud M., Garnero P., Dayer J. M., Guerne P. A., Fossiez F. and Miossec P. (2000) Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis. Cytokine **12:** 1092–1099
116 Benderdour M., Tardif G., Pelletier J. P., Di Battista J. A., Reboul P., Ranger P. et al. (2002) Interleukin 17 (IL-17) induces collagenase-3 production in human osteoarthritic chondrocytes via AP-1 dependent activation: differential activation of AP-1 members by IL-17 and IL-1beta. J. Rheumatol. **29:** 1262–1272
117 Chabaud M., Lubberts E., Joosten L., van Den B. W. and Miossec P. (2001) IL-17 derived from juxta-articular bone and synovium contributes to joint degradation in rheumatoid arthritis. Arthritis Res. **3:** 168–177
118 Cai L., Yin J. P., Starovasnik M. A., Hogue D. A., Hillan K. J., Mort J. S. et al. (2001) Pathways by which interleukin 17 induces articular cartilage breakdown in vitro and in vivo. Cytokine **16:** 10–21
119 Lubberts E., Joosten L. A., Oppers B., van Den B. L., Coenen-de Roo C. J., Kolls J. K. et al. (2001) IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. J. Immunol. **167:** 1004–1013
120 Bush K. A., Farmer K. M., Walker J. S. and Kirkham B. W. (2002) Reduction of joint inflammation and bone erosion in rat adjuvant arthritis by treatment with interleukin-17 receptor IgG1 Fc fusion protein. Arthritis Rheum. **46:** 802–805
121 Burchill M. A., Nardelli D. T., England D. M., DeCoster D. J., Christopherson J. A., Callister S. M. et al. (2003) Inhibition of interleukin-17 prevents the development of arthritis in vaccinated mice challenged with *Borrelia burgdorferi*. Infect. Immun. **71:** 3437–3442
122 Lubberts E., van Den B. L., Oppers-Walgreen B., Schwarzenberger P., Coenen-De Roo C. J., Kolls J. K. et al. (2003) IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappaB ligand/osteoprotegerin balance. J. Immunol. **170:** 2655–2662
123 Nakae S., Saijo S., Horai R., Sudo K., Mori S. and Iwakura Y. (2003) IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. Proc. Natl. Acad. Sci. USA **100:** 5986–5990
124 Fujino S., Andoh A., Bamba S., Ogawa A., Hata K., Araki Y. et al. (2003) Increased expression of interleukin 17 in inflammatory bowel disease. Gut **52:** 65–70
125 Nielsen O. H., Kirman I., Rudiger N., Hendel J. and Vainer B. (2003) Upregulation of interleukin-12 and -17 in active inflammatory bowel disease. Scand. J. Gastroenterol. **38:** 180–185
126 Bamba S., Andoh A., Yasui H., Araki Y., Bamba T. and Fujiyama Y. (2003) Matrix metalloproteinase-3 secretion from human colonic subepithelial myofibroblasts: role of interleukin-17. J. Gastroenterol. **38:** 548–554
127 Andoh A., Hata K., Araki Y., Fujiyama Y. and Bamba T. (2002) Interleukin (IL)-4 and IL-17 synergistically stimulate IL-6 secretion in human colonic myofibroblasts. Int. J. Mol. Med. **10:** 631–634
128 Lock C., Hermans G., Pedotti R., Brendolan A., Schadt E., Garren H. et al. (2002) Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat. Med. **8:** 500–508
129 Matusevicius D., Kivisakk P., He B., Kostulas N., Ozenci V., Fredrikson S. et al. (1999) Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. Mult. Scler. **5:** 101–104
130 Pelidou S. H., Zou L. P., Deretzi G., Oniding C., Mix E. and Zhu J. (2000) Enhancement of acute phase and inhibition of chronic phase of experimental autoimmune neuritis in Lewis rats by intranasal administration of recombinant mouse interleukin 17: potential immunoregulatory role. Exp. Neurol. **163:** 165–172
131 Loong C. C., Hsieh H. G., Lui W. Y., Chen A. and Lin C. Y. (2002) Evidence for the early involvement of interleukin 17 in human and experimental renal allograft rejection. J. Pathol. **197:** 322–332
132 Hsieh H. G., Loong C. C., Lui W. Y., Chen A. and Lin C. Y. (2001) IL-17 expression as a possible predictive parameter for subclinical renal allograft rejection. Transpl. Int. **14:** 287–298
133 Woltman A. M., de Haij S., Boonstra J. G., Gobin S. J., Daha M. R. and van Kooten C. (2000) Interleukin-17 and CD40-ligand synergistically enhance cytokine and chemokine production by renal epithelial cells. J. Am. Soc. Nephrol. **11:** 2044–2055
134 Antonysamy M. A., Fanslow W. C., Fu F., Li W., Qian S., Troutt A. B. et al. (1999) Evidence for a role of IL-17 in alloimmunity: a novel IL-17 antagonist promotes heart graft survival. Transplant. Proc. **31:** 93
135 Antonysamy M. A., Fanslow W. C., Fu F., Li W., Qian S., Troutt A. B. et al. (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:** 577–584
136 Tang J. L., Subbotin V. M., Antonysamy M. A., Troutt A. B., Rao A. S. and Thomson A. W. (2001) Interleukin-17 antagonism inhibits acute but not chronic vascular rejection. Transplantation **72:** 348–350
137 Numasaki M., Fukushi J., Ono M., Narula S. K., Zavodny P. J., Kudo T. et al. (2003) Interleukin-17 promotes angiogenesis and tumor growth. Blood **101:** 2620–2627
138 Kato T., Furumoto H., Ogura T., Onishi Y., Irahara M., Yamano S. et al. (2001) Expression of IL-17 mRNA in ovarian cancer. Biochem. Biophys. Res. Commun. **282:** 735–738

139 Hirahara N., Nio Y., Sasaki S., Minari Y., Takamura M., Iguchi C. et al. (2001) Inoculation of human interleukin-17 gene-transfected Meth-A fibrosarcoma cells induces T cell-dependent tumor-specific immunity in mice. Oncology **61:** 79–89
140 Hirahara N., Nio Y., Sasaki S., Takamura M., Iguchi C., Dong M. et al. (2000) Reduced invasiveness and metastasis of Chinese hamster ovary cells transfected with human interleukin-17 gene. Anticancer Res. **20:** 3137–3142
141 Benchetrit F., Ciree A., Vives V., Warnier G., Gey A., Sautes-Fridman C. et al. (2002) Interleukin-17 inhibits tumor cell growth by means of a T-cell-dependent mechanism. Blood **99:** 2114–2121
142 Aggarwal S. and Gurney A. L. (2002) IL-17: prototype member of an emerging cytokine family. J. Leukoc. Biol. **71:** 1–8