

3.3 Cytokines 236

3.3 Cytokines 236

ESSENTIALS Cytokines are small glycoprotein mediators that are involved in every facet of immune effector function and regulation, and moreover serve to integrate immune function with other physiologic processes (e.g. metabolism, neurologic function). More than 200 cytokines have been identified. They can (1) function through binding to specific receptors that in turn signal via complex transduction pathways to regulate gene expression, thereby mediating positive and negative regulatory activities; (2) operate as soluble mediators in the extra-cellular domain or within cells, where they may also traffic to the nucleus and exhibit dual function as transcriptional regulators; (3) be expressed initially on the cell membrane, where they may exert effector function directly in cell-cell interactions, or from which they can be subsequently cleaved to yield bioactive soluble molecules, thereby mediating autocrine and paracrine activities around their cellular source. The innate immune response—this is designed to offer immediate defence and comprises particular leucocyte lineages including neutrophils, mast cells, eosinophils, monocytes/macrophages, and natural killer cells. It is regulated by cytokines such as the type I interferons (IFN), tumour necrosis factor (TNF) α , interleukin (IL) 1 superfamily, and IL-6 superfamily, which are essential for the integrity of the innate immune response. Additional cytokines such as IL-10 and tissue growth factor (TGF) β promote resolution of immune responses and mediate healing of tissue damage, but they can play a substantial role in chronic inflammation when improperly down regulated. Adaptive immune responses—these are an evolutionary refinement to facilitate recall responses to specific antigens derived from invading organisms or damaged self-tissue. Professional antigen-presenting cells are activated by cytokines such as IFNs, TNF α , and IL-1 α to increase antigen uptake, processing, and presentation to naive T cells, and—critically—dendritic cells use cytokines to define the phenotype of subsequent T-cell responses: Th1 (IFN γ predominant); Th17 response (IL-17A, IL-17F, IL-22 predominant); and Th2 (IL-4, IL-5, IL-13 predominant). Regulatory T-cell subsets may also be activated in this phase by cytokines. Activation of host-tissue cell types—cytokines mediate many effector functions in the immune system by action on host-tissue cell types, including (1) activation of endothelial and lymphendothelial cells to express adhesion molecules and to alter their permeability properties to facilitate induction and resolution of inflammation; (2) regulation of differentiated tissue specific cells, which can contribute indirectly to host-tissue defence; (3) regulation of host-tissue function in the context of inflammation (e.g. by facilitating metabolic responses to sustain energy requirements for host-defence responses). Clinical context—understanding of the cytokine network has increasing importance in clinical practice with the advent of therapeutic strategies that target particular cytokines with exquisite specificity using biological agents, leading to remarkable advances in the treatment of inflammatory disorders (e.g. anti-TNF therapy in rheumatoid arthritis).

and anti-IL-17A in psoriasis). The therapeutic potential in their manipulation has not yet been maximized and the future will hold remarkable advances as these molecular networks give up their secrets to provide for highly specific and well-tolerated interventions. Introduction In higher organisms, the immune system has evolved to provide flexible and comprehensive host defence against microbial organisms. It also plays a critical role in recognition of, and immediate response to, altered integrity of self-tissues, for example as occurs in trauma or neoplasia. The immune system itself comprises a complex interaction between cells of discrete lineage and function that must act in a cooperative manner to achieve satisfactory, long-lived protection with minimal damage to the host. Cytokines are small glycoprotein messengers (8–40 kDa) that facilitate regulation and effector function of cells in an autocrine or paracrine manner. Typically, cytokines exhibit functional activities that are of critical importance in the immune system, but also mediate wider effects across a range of tissues. Cytokines thus also play a role in a variety of normal physiological and metabolic processes. The field of cytokine biology has expanded considerably in the last decade with the recognition of large numbers of moieties and the advent of effective therapeutics based on cytokine targeting, particularly in inflammatory diseases. 3.3 Cytokines

3.3 Cytokines 237 Classification of cytokines Cytokines were originally discovered and defined on the basis of their functional activities observed in bioassays (e.g. macrophage activating factor, lymphocyte activating factor). Subsequent advances in molecular biology, bioinformatics, and recently the Human Genome Project have facilitated discovery of large numbers of cytokines, posing considerable challenges in resolving their individual and synergistic functions in complex tissues in health and disease. In the absence of a unified classification system, cytokines may be variously identified by:

- numerical order of discovery (e.g. the interleukins (IL): currently IL-1 to IL-40)
- specific functional activity (e.g. tumour necrosis factor, TNF; granulocyte colony stimulating factor, G-CSF)—this is usually an inaccurate descriptor of the potential activities of a given cytokine and therefore interpretation of function from such nomenclature should be cautious.
- on the basis of predominant primary cell or tissue of origin (monokine = monocyte derived; lymphokine = lymphocyte derived; adipocytokine = adipose tissue derived)
- structural homologies shared with related molecules

Cytokines may thus be considered in ‘family groups’ that share sequence similarity and also exhibit homology and some promiscuity in their reciprocal receptor systems. Note that they need not exhibit functional similarity despite common structural domains or features. These so-called ‘cytokine superfamilies’ often contain regulatory cell membrane receptor–ligand pairs that use common structural motifs in diverse immune functions in higher mammals, reflecting evolutionary pressures. This is best exemplified in the IL-1/IL-1 receptor superfamily that contains cytokines such as IL-1 β , IL-1 α , IL-1 receptor antagonist, IL-1 F5–F10, IL-18, IL-33, IL-36, and IL-37 which mediate physiological and host-defence function. IL-1 receptor signalling cascades share remarkable similarities with the signalling pathways induced by toll-like receptors (TLR) and NOD-like receptors (NLR), a series of mammalian pattern-recognition molecules with a crucial role in recognition of microbial species early in innate responses. These common motifs allow integration of responses at the signal transduction level between cytokine and other immune receptor systems to allow fine-tuning of responses over time. Increasingly, cytokines might also be usefully organized on the basis of their cooperative functional properties and thereby assigned to a given immunologic process (e.g. T-cell activation, T-cell differentiation, or tissue repair). One cytokine can contribute to several activities and one cytokine superfamily can by corollary contribute to many discrete immunologic processes. Basic cytokine biology See Fig. 3.3.1. Synthesis, expression, and regulation Cytokines can be produced by almost every

leucocyte and host tissue cell type (see next). Cytokines can be secreted or expressed as cell membrane-bound proteins, or may be processed into cytosolic forms that can traffic intracellularly, even to the nucleus where they can act as transcriptional regulators. Thus, cytokines mediate autocrine function either through release or membrane expression and immediate receptor ligation on the source cell, or intracellularly within the source cell. Alternatively, cytokines operate in a paracrine manner, allowing cellular communication beyond that facilitated by local cell-cell contact. The distance over which cytokines can mediate effects is unclear and probably depends on many factors, not least the site of synthesis and the extracellular matrix components of a given tissue: in silico models predict meaningful bioactivity for many cytokines no further than a 40 μm diameter from the source cell due to physicochemical considerations of the peptide structure itself, extracellular matrix binding (e.g. to heparan sulphate), enzymatic degradation, or the presence of soluble receptors or cytokine-binding proteins. Some cytokines, however, clearly exhibit systemic activities (e.g. IL-1 β promotes pyrexia via either local expression or circulation to the central nervous system, and IL-6 induces the acute-phase response by circulating to the liver). To facilitate such broader effects, IL-6 receptor also circulates as a soluble entity (soluble IL-6 receptor) and thereby confers on this cytokine the ability to activate any cell expressing its other cognate receptor (gp130) since it can preform IL-6/IL-6R complexes in the circulation that can directly bind and activate cells via gp130. One implication of this is that simple measurement of a cytokine concentration alone may not reflect its functional contribution at the biologic level unless other functioning components are also assessed, which has confounded efforts to use cytokines as biomarkers in clinical development programmes. The triggers to cytokine release are diverse and depend upon the cell type and tissue concerned (Box 3.3.1). Many of these triggers can also be used ex vivo to study cytokine function, together with a variety of surrogate stimuli such as chemical entities, including phorbol esters, calcium ionophores, lectins and receptor-specific agonistic antibodies (e.g. anti-CD3/CD28 to mimic T-cell costimulatory activation) and microbial derived derivatives (e.g. LPS, BLP). Detailed studies using such agents in vitro together with in vivo observations using gene knock-in and knock-out mice have unravelled many layers of regulation for cytokine production. Since they exhibit such potent effects in immune function, it is unsurprising that these pathways are tightly regulated. Transcriptional and post-transcriptional regulation Transcription of a cytokine gene depends upon the recruitment of usually multiple transcription factors (TFs) to the cytokine gene promoter region. TF binding allows numerous signal pathways to regulate cytokine expression. Some transcription factors (e.g. NF- κ B, activator protein-1, AP-1; nuclear factor of activated T cell, NF-AT) appear to have particular importance in cytokine regulation in disease states. However, there are many other TF binding sites in most cytokine genes and increasingly the functional organization of chromatin structures and transcriptional machinery is recognized to be rather different depending upon the cell type and cytokine studied. The recognition and manipulation of cytokine transcription is both an area of enormous complexity at present, but also great therapeutic opportunity to achieve context-specific inhibition of cytokine production (e.g. inflammatory vs. protective cytokine release). Transcription factors that define cell types/differentiation

238 SECTION 3 Cell biology status and that are directly cytokine regulated offer particular hope in this regard (e.g. t-bet, ror-c that regulate Th1 and Th17 cell differentiation, respectively). Sequence polymorphism within cytokine promoters offers potential for differential cytokine expression between individuals that could confer selective advantage against infection, but could also increase susceptibility to, or progression of, autoimmunity or chronic inflammation. Intronic

single nucleotide polymorphisms (SNPs) elsewhere in the cytokine gene structure are also likely to be of importance although they are in general ill-defined at present. Post-transcriptional regulation is important in determining longevity of cytokine expression. This may operate by promoting translational initiation, mRNA stability, and polyadenylation. AU-rich elements within the 5' or 3' untranslated regions (UTR) of cytokine mRNA are crucial for stability. Alternatively, cytokines may generate stable mRNA *a priori* to facilitate subsequent rapid response in tissues. By this means cytokines generated by distinct processing of this mRNA can be rapidly synthesized and trafficked to cytosolic or extracellular domains as required. Finally, the cytosolic activity of microRNA species provides a further level of cytokine regulation. These recently recognized small RNA sequences have the capacity to bind to mRNA and down-regulate translation to protein. Myriad microRNAs have now been identified, each considered capable of regulating up to 20 mRNAs, usually in a negative direction. Genetic targeting of such microRNAs *in vivo* has led to remarkable effects on global immune function mediated through altered cellular differentiation and cytokine release. In terms of cytokine regulation there has been particular interest in the biology of miR146a and miR155, though many others are now emerging.

Tissue structure
 Autoantibody synthesis
 macrophage
 Matrix damage
 Fibroblast
 Adaptive response
 m DC
 p DC
 B cell
 GC formation
 Innate response
 Mast cell
 Endothelial cell, smooth muscle cell activation/
 angiogenesis
 adipocyte
 Neutrophil
 Osteoclast precursor
 Chondrocyte
 Atherogenesis
 Metabolic syndrome
 Inflammation
 BlyS
 APRIL
 IL-6
 IL-10
 LTβ
 CXCL13
 CCL21
 IL-15
 TNF
 IL-6
 IFNγ
 IL-18
 Cell-contact
 IL-23
 IL-6
 TGFβ
 IL-12
 IL-15
 IL-18
 IFNα/β
 IL-15
 IL-18
 IL-1
 RANKL
 TNF
 M-CSF
 IL-17
 Cadherin-11
 Adiponectin
 TNF
 IL-6
 IL-15
 IL-1
 resistin
 VEGF
 bFGF
 IL-1
 TNF
 IL-6
 TGFβ
 IL-17
 IL-32
 IL-1
 IL-18
 TNF
 GM-CSF
 IL-17
 Th17 / Th1 cell
 Treg cell
 IL-10
 TGFβ
 IL-17
 RANKL
 Cell-contact
 TLR, PAR, FcR

Points of cytokine synergy / cooperation with: Co-stimulation
 Plasma cell

Fig. 3.3.1 Cytokines mediate pleiotropic activities within complex cellular systems. Cytokines form coordinated networks that regulate multiple cellular interactions locally and in turn promote systemic responses. This is particularly well-defined in the pathogenesis of rheumatoid arthritis. The principle relationships existing between cellular lineages are consistent across a range of inflammatory disorders. The figure highlights the multifaceted roles played by cytokines in the range of manifestations of disease. The activities are broadly defined on the basis of their activities in promoting adaptive or innate immune function. In the novel immune response these will occur sequentially. In the context of chronic inflammation, or persistent recall responses, the two arms of the immune system will likely overlap allowing for considerable cross-talk and interlinked function for cellular components, but particularly in the cytokine network. Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Immunol. Cytokines in the pathogenesis of rheumatoid arthritis. McInnes IB, Schett G. 2007 Jun; 7(6):429-42, © 2007.

3.3 Cytokines 239 Post-translational regulation Cytokine production is regulated by post-translational modifications. Patterns of glycosylation are important for cytokine function in the extracellular domain and may also regulate intracellular trafficking. Modified leader sequences can alter intracellular trafficking of cytokines. Moreover, some cytokines are translated without functional leader sequences. Their secretion depends on nonconventional secretory pathways that are thus far poorly understood but may include ion channels, chaperone proteins, or specific membrane pores. Enzymatic activation of preformed procytokines is common. Such enzymatic cleavage is often conducted within an assembly of proteins (e.g. the inflammasome) designed to carefully coordinate and regulate the amount and duration of active cytokine release. A variety of

enzymes are implicated in these processes including caspases, the serine proteases, proteinase 3, and elastase, and adamolysin family members. Enzyme cleavage pathways operate both within and outside cells, providing for extracellular cytokine activation. Thus, cell membrane enzymes serve to cleave membrane-expressed cytokine to generate soluble cytokines. In summary, extensive molecular machinery exists to tightly regulate not only the production and stability of cytokine mRNA, but also its translation and cellular expression and distribution. Mediation of cytokine effects

Cytokines mediate their effects primarily via the binding to a receptor(s) or combination of receptors that is (are) expressed on the membrane or cytosol of a target cell. Like cytokines, and arising from similar evolutionary mechanisms, receptors for cytokines exist in structurally related superfamilies (Fig. 3.3.2). Cytokine receptors thus comprise high-affinity molecular signalling complexes that facilitate cytokine-mediated communication. Such complexes often include heterodimeric or heterotrimeric structures that use unique, cytokine-specific recognition receptors together with common receptor chains shared across a cytokine superfamily. The common γ chain is used by receptor complexes of many α -helix cytokines (e.g. IL-2, IL-7, IL-15, IL-21) and the gp130 receptor is similarly utilized by IL-6 and many homologues. Similar promiscuity across cytokine receptors is shown by the intracellular molecules used to transduce the signal to the nucleus (e.g. JAK/STAT pathways). Single signal molecules in turn can function on behalf of many receptors, although subtle differences may operate as to precise dimerization or phosphorylation events for any given receptor leading to specificity of the response. The JAK/STAT receptor signalling complex is particularly useful to demonstrate the very broad range of functions that can be achieved, with the potential for fine tuning based on a relatively limited array of receptor and transduction components. Cytokines and their receptors can adopt a variety of orientations to mediate their effector functions. Membrane receptors, with intracellular signalling domains intact, can transmit signals to the target cell nucleus following soluble cytokine binding and thereby promote effector function. Membrane receptors may bind cell membrane cytokines, facilitating cross-talk between adjacent cells that can alter the behaviour of both participating cells, particularly if a cytokine exhibits the potential to 'reverse signal' (the property to send a signal back into the producer cell via membrane cytokine expression). Membrane-bound and soluble cytokines may thus promote distinct functions. Cytokine receptor-cytokine complexes may also operate in trans, whereby component parts of the ligand-receptor complex are derived from adjacent cells. This renders target cells capable of responses to a cytokine when they only express a part of the necessary cytokine receptor complex. Receptors also exist in soluble form, derived either from alternative mRNA processing to generate receptor-lacking transmembrane or intracellular domains, or by enzymatic cleavage of receptor from the cell surface. Soluble receptors can antagonize cytokine function, or preform complexes with cytokine to promote subsequent ligand-receptor assembly on the target cell membrane, and thereby enhance function as another example of trans signalling. Furthermore, soluble receptors can deliver cytokine to the cell membrane via ligand passing, in essence the donation of a cytokine from one receptor to another on the surface of a cell to confer sequential function.

Box 3.3.1 Factors that regulate cytokine production

- Cytokines (forming amplificatory and regulatory loops)
- Cell-cell contact (e.g. lectins, integrins, Ig superfamily members)
- Immune complexes/autoantibodies
- Complement activation products
- Microbial species and their soluble products (particularly via TLR/NLR pathways)
- Reactive oxygen and nitrogen intermediates
- Trauma
- Sheer stress and barotrauma
- Ischaemia
- Radiation
- Ultraviolet light
- Extracellular matrix components
- DNA (mammalian or microbial) and RNA (including microRNA and lncRNA)
- Heat-shock proteins

Fig. 3.3.2 Cytokine receptors and the intracellular molecules used to transduce

the signal.

240 SECTION 3 Cell biology These details of how cytokines mediate their effects are crucially important in devising effective therapeutic cytokine inhibitors. The homeostasis of cytokine release and function is maintained by this network arrangement. An inhibitor that blocks only part of the cytokine receptor interaction may be ineffective or, worse, paradoxically enhance effects (Fig. 3.3.2). Finally, although in general cytokines bind only their cognate receptor, there appears to be some plasticity in the system since close cross-communication on the cell membrane between seemingly unrelated cytokine receptor systems also occurs, thereby allowing a cell to integrate a variety of external stimuli to optimize signalling pathways. This allows cells to constantly sense and respond to complex changes in the local environment delivered by many cytokines either simultaneously or in sequence. Activities of principal cytokines Members of the major cytokine superfamilies are listed in Table 3.3.1. Further hierarchical relationships exist within and between superfamilies, reflecting the ancestral genes from which cytokines have been derived. The diversity and density of data now available to describe the activities of individual cytokines are beyond the scope of this text, but the following paragraphs summarize the activities of selected cytokines implicated in inflammatory disorders. TNF superfamily Tumour necrosis factor Tumour necrosis factor (TNF) is the prototypic proinflammatory cytokine of this superfamily. It is produced by a wide variety of immune cell types, particularly macrophages, T and B lymphocytes, and neutrophils, but is also released by tissue cell types including keratinocytes, fibroblasts, glial cells, and smooth muscle cells. It comprises a heterotrimer (each subunit of 26 kDa) that binds to either of two receptors TNF receptor I (p55) or TNF receptor II (p75). TNF and its receptors are synthesized as membrane proteins that can be cleaved to soluble form by the activity of members of the ADAMs family of enzymes. Downstream signalling is mediated via MAPK and NF- κ B, and can if appropriate involve recruitment of death domains to facilitate apoptosis in target cells. TNF sits in a pivotal position in many inflammatory cytokine networks. Thus, inhibition of TNF in inflammatory tissues *in vitro*, such as those derived from inflammatory synovitis, psoriatic skin, or inflammatory bowel disease mucosal biopsies leads to down-regulation of many other inflammatory cytokines such as IL-6 and IL-8, and of the production of many inflammatory chemokines. This 'cytokine hierarchy' is considered to explain in part the efficacy of single cytokine targeting in chronic disease states. The precise effects of TNF receptor binding depend upon the lineage and activation status of the target cell. TNF induces monocyte activation and maturation and promotes chemokinesis, release of reactive oxygen and nitrogen intermediates (ROI/RNI), and prostaglandin/leukotriene production. Similarly, polymorphonuclear leucocytes are primed and induced to oxidative burst by TNF. Effects on T cells are predominantly regulatory such that long-standing exposure to TNF leads to relative hypofunction of T cells and impaired T-cell receptor signalling. Indeed, TNF blockade in humans can lead to enhanced T-cell autoreactivity. TNF is a critical activator of tissue cells. Thus, endothelial cells (vascular) and lymphendothelial cells (lymphatics) are induced to express high levels of adhesion molecules and chemokines upon TNF exposure. The net effect of this is to increase cellular trafficking into and out of inflammatory lesions. It further promotes vascular permeability and is directly implicated in the hypotension and oedema associated with septic shock. Local effects are also mediated upon nociception such that TNF increases pain sensation via modulated local neurotransmitter release. Systemic metabolic effects can promote cachexia and altered lipid metabolism in adipose tissues. Mice in which TNF is transgenically overexpressed develop spontaneous autoinflammatory diseases including inflammatory arthritis and inflammatory bowel disease. Targeting TNF is effective in numerous disease states, particularly

rheumatoid arthritis, Crohn's disease, and psoriasis, providing formal proof of concept of a pivotal role for this cytokine in pathogenesis. Lymphotoxin Lymphotoxin is a 22 to 26 kD cytokine that shares broad inflammatory properties with TNF but is particularly and additionally Table 3.3.1 Members of the major cytokine superfamilies Cytokine family/activity Key membersa TNF-like TNF, lymphotoxin, BlyS, APRIL, RANKL, TWEAK IL-1-like IL-1 α , IL-1 β , IL-1Ra, IL-18, IL-33, IL-36 α , β , γ , IL-37, IL-38, IL-36Ra IL-6-like IL-6, oncostatin M, leukaemia inhibitory factor, IL-11, IL-31, cardiotrophin-1, cardiotrophin-like cytokine ciliary neurotrophic factor IL-12-like IL-12, IL-23, IL-27, IL-35 IL-10-like IL-10, IL-19, IL-20, IL-22, IL-24 Growth factors TGF β , BMPs, PDGF Angiogenesis VEGF, bFGF, endostatin Colony stimulating factors G-CSF, M-CSF, GM-CSF Adipocytokines adiponectin, resistin, IL-17 family IL-17A, IL-17F, IL-17B, IL-17C, IL-17E aThe cytokines shown for each family are examples, not an exhaustive list.

3.3 Cytokines 241 implicated in structural organization of the immune system. Thus, formation of germinal centres in lymph nodes and spleen and the creation of 'ectopic' germinal centres (i.e. formed outwith lymphoid organs) in chronically inflamed tissues is particularly regulated by lymphotoxin, together with the chemokines CCL13 and CXCL21. B lymphocyte stimulator protein B lymphocyte stimulator protein (BlyS; also known as B-cell activating factor, BAFF) and a proliferation inducing ligand (APRIL) are two further members of the TNF superfamily that regulate B-cell function. BlyS and APRIL can be synthesized by monocytes, T cells, dendritic cells, fibroblasts, and some tumour cells. Their primary activity lies in supporting B-cell maturation and activation transduced via BlyS receptor and TACI. Following antigen-driven B-cell activation, BlyS and APRIL promote isotype switching and immunoglobulin secretion and delay B-cell apoptosis. Effects beyond B cells have been observed including T-cell costimulation and tumour proliferation. Receptor activator of NF- κ B ligand Receptor activator of NF- κ B ligand (RANKL) was identified as a cytokine (35 kDa) promoting dendritic cell-T-cell interactions but is now primarily recognized as a critical regulator of bone homeostasis. RANKL is produced by monocytes, T cells, osteoblasts, and stromal cells and binds to RANK, its cognate receptor. RANKL mediates effects in physiological bone remodelling by promoting maturation of osteoclast precursors to yield osteoclasts that are fully functional for resorption of calcified tissues. Production by osteoclasts facilitates integrated resorption and new bone formation to maintain structural integrity and morphology of bone. In inflammatory states, RANKL expression is increased leading to increased osteoclast maturation and effector function leading to net bone loss. RANKL exhibits synergistic effects with TNF, IL-1 β , IL-17, and IL-6 in this regard. Osteoprotegerin (OPG) is a 55 kDa soluble decoy receptor for RANKL that acts as a competitive inhibitor to limit the activity of RANKL. OPG-deficient mice exhibit significant osteoporosis and transgenic mice are osteopetrotic, suggesting a critical role in physiologic bone remodelling. The balance between RANKL and OPG synthesis defines the net resorptive activity of bone and is dysregulated in inflammatory states leading to systemic bone loss (i.e. osteoporosis). An increased inflammatory milieu usually increases the RANKL/OPG ratio of expression leading to accelerated local bone loss, designated 'erosions'. These are particularly prevalent in rheumatoid arthritis and psoriatic arthritis, but also occur in septic arthritis. This can also mediate systemic bone loss and predispose patients with chronic inflammatory diseases to osteoporosis. IL-1 superfamily The IL-1 superfamily contains a variety of moieties involved in local and systemic regulation of immune responses that share structural homology. This family has recently expanded and now comprises a group of moieties with agonist activity that is generally proinflammatory, including IL-1 α , IL-1 β , IL-18, IL-33, IL-36 α , IL-36 β , and IL-36 γ . IL-37 is an agonist that appears to predominantly anti-inflammatory in its net effects. There are now three receptor

antagonists that have been recognized, designated IL-1Ra, IL-36R α , and IL-38. IL-1 Most is known about IL-1 α and IL-1 β that are synthesized as promolecules of approximately 35 kDa which are in turn cleaved by caspase 1 within the inflammasome to yield active 18 kDa cytokines. IL-1Ra is a homologue of IL-1 α and IL-1 β that competes with these agonists for receptor binding. The IL-1 cytokines effect function via binding to a heterodimeric receptor comprising IL-1 receptor I (IL-1RI) and IL-1 receptor accessory protein (IL-1RAcP). IL-1RII is a further receptor that has decoy function. IL-1 cytokines signal through a canonical signal pathway that they share with the TLR superfamily. Via a series of protein interactions and kinase dependent events, this pathway leads to NF- κ B activation and inflammation related gene activation. IL-1 α and IL-1 β are differentiated by the primarily membrane expression of the former which also retains activity as a full-length promolecule. Their functions are, however, rather similar, reflecting the shared receptor components (and are designated 'IL-1' hereafter). Thus they promote monocyte activation, cytokine and ROI/RNI release, and prostaglandin production. They further drive fibroblast activation, collagen synthesis, prostaglandin release, and proliferation. IL-1 is a potent inducer of endothelial cell activation and adhesion molecule expression, osteoclast maturation, and activation in synergy with RANKL, chondrocyte activation, catabolism, and matrix degradation via metalloproteinase production. IL-1 is a potent pyrogen commensurate with the fever syndromes that arise when genetic abnormalities occur in IL-1 regulation (e.g. Muckle Wells syndrome/cold autoinflammatory disorders). Such syndromes are accordingly amenable to therapeutic IL-1 blockade using, for example, synthetic IL-1Ra (anakinra) or anti-IL-1 antibodies. IL-1 is also implicated in the pathogenesis of a variety of common chronic inflammatory diseases including rheumatoid arthritis, ankylosing spondylitis, and psoriasis. Its hierarchical position in inflammation cascades relative to TNF is unclear since targeting with IL-1Ra in practice has proved disappointing in most diseases in which TNF blockade is effective. However, IL-1 has found a favourable role as a target in diseases associated with excess inflammasome activation, particularly gout. There is also interest in its metabolic activities in type II diabetes in which therapeutic targeting may be beneficial. Finally, high levels of IL-1 expression within the central nervous system may regulate several central pathways implicated in cognition and mood state. IL-18 IL-18 is an innate response cytokine produced by monocytes, fibroblasts, neutrophils, and dendritic cells as a 33 kDa promolecule that can be cleaved by the actions of caspase 1 in the NALP3 inflammasome to an 18 kDa active moiety. IL-18 activates a heterodimeric receptor (IL-18R α /IL-18R β) and is antagonized in vivo by soluble IL-18R α and by a distinct IL-18 binding protein family that contains several members generated by alternative mRNA splicing. IL-18 activates neutrophils to promote maturation, chemotaxis, ROI/RNI production, and cytokine/chemokine release. It also drives NK-cell activation, and monocytes/macrophage maturation and activation. Its primary effects are likely mediated on driving T-cell differentiation towards a mainly Th1 phenotype in synergy with IL-12. IL-18 is expressed at high levels in rheumatoid arthritis, psoriasis, and

242 SECTION 3 Cell biology inflammatory bowel disease in human tissues and rodent models and has net proinflammatory function therein. Very high systemic levels have been reported in Still's disease (juvenile and adult onset) and in systemic leukophagocytosis syndromes. Recently effects on hepatocytes and adipocytes have been reported for IL-18. Their net effect on metabolism and accrued vascular risk is unclear since epidemiological and several in vitro studies suggest that IL-18 can promote atherogenic risk and metabolic syndrome whereas in vivo studies mainly in IL-18-deficient animals suggest that IL-18 may be protective in this regard. This may offer therapeutic

opportunity in due course. IL-33 is produced mainly by fibroblasts, and is synthesized as a 33 kDa protein that is cleaved by enzyme pathways as yet undefined. Its effects function via ST2L and IL-1RAcP binding and signals via the canonical IL-1 receptor pathway. It also acts within the nucleus of the synthesizing cell as a transcriptional repressor by virtue of a direct DNA binding domain. IL-33 activates Th2 cell and ILC2, iNKT cell differentiation and expansion. It directly activates mast cell and eosinophil activation and cytokine production. As such, the effects attributed to IL-33 are mainly in allergy and anaphylaxis. It can also activate B cells, macrophages, and neutrophils. Recently a role for IL-33 in promoting immune homeostasis in the gastrointestinal mucosa has been suggested via promotion of regulatory T-cell functions. IL-36 and IL-37 IL-36 α,β,γ may be considered together at this time. IL-36 α,β,γ are produced by macrophages, dendritic cells, and some lymphocyte subsets and also by epithelial cells and synovial fibroblasts rendering them of interest in a variety of chronic disease states. Their effects can be antagonized by IL-36Ra. Via activation of keratinocytes IL-36 is implicated in psoriasis, and also has been shown to promote neutrophilic pulmonary disease. IL-37 exists in five splice variants, but at this time its functions are relatively poorly understood, although in general they appear to be anti-inflammatory. Type I Interferons Interferons were first described more than half a century ago and are of pivotal importance in primary host defence, but increasingly have been attributed effector roles across a range of cancers and inflammatory diseases. Type I interferons comprise IFN α , (of which there are 13 subtypes in humans that share broad homology), IFN β , and others including IFN ϵ , IFN τ , IFN κ , and IFN ω . They are synthesized by many immune and tissue cells as part of the primary response to viral infection, usually induced by pattern-recognition receptors (PRR) that 'sense' a variety of shared structures derived from microbial species, or components of tissue damage (e.g. soluble DNA, RNA). PRR in turn activate IFN-regulatory family transcription factors leading to IFN α and IFN β synthesis. Type I IFNs in turn bind their receptor IFNAR1/IFNAR2, leading to activation of interferon stimulated response elements in many promoters that permits induction of expression of many so-called IFN-stimulated genes (ISGs). The functional consequences are complex. Type I IFN serve to restrict viral synthesis within infected cells and transmission to adjacent tissue cells. They also mediate wider immune modulatory effects across many cells of the innate immune response. Specifically, they activate dendritic cells and monocytes, CD4 and CD8 T cells, NK cells, and B cells. Thus, they offer both elements of host defence but also host-tissue damage, with the latter becoming dominant in the context of chronic inflammatory diseases. For example, in rheumatoid arthritis and especially in systemic lupus erythematosus, type I IFN responses are predominant and present in many patients. Recently it was recognized that type I IFNs are also up-regulated within tumour microenvironments, both within tumour cells and infiltrating immune effector cells such that they can regulate the autocrine and paracrine regulation of immune surveillance. Based on these varied clinical observations it is unsurprising that there remains intense interest in the use of type I IFNs both as therapeutics per se (e.g. as established agents for the treatment of multiple sclerosis), and also as immune target in the context of clinical trials (e.g. the development of anti-IFN and anti-IFNAR antibodies in systemic lupus erythematosus). Moreover, the presence of ISGs is also considered to carry rich potential to serve as biomarkers in chronic inflammatory diseases (e.g. high ISG expression is associated with low responses to rituximab in rheumatoid arthritis).

Type II and type III interferons The type II interferon class comprises only IFN γ that has a well-established role in the regulation of monocyte and dendritic cell activation arising as a product of TH1 cells, ILC1, and NKT cells. The wider role of IFN γ is discussed later here in the context of cellular activation and T-cell function. Type III interferons include IFN λ 1, IFN λ 2, and IFN λ 3 (also designated as IL-28), but little is currently known about their relevant effector biology. Other

cytokine activities Regulation and mediation of T-cell function T cells of CD4 + and CD8 + lineages are functionally defined on the basis of their release of effector cytokines. Th1 cells (defined by t-bet transcription factor expression) release IFN γ and TNF α and promote granuloma formation and host defence to intracellular organisms. Th1 cell formation is driven by IL-12 and IL-18 together with relative absence of TGF β , IL-4, and IL-17. IFN γ is a 20 to 25 kDa molecule that has pleiotropic functions including priming and activation of neutrophils, activation of macrophages particularly to induce cytotoxic pathways, and activation of NK cells. However, IFN γ also promotes tissue repair and as such is an example of the multifunctional potential in cytokines that must be elucidated with care prior to therapeutic intervention. Inherited deficiencies in the IFN γ /IFN γ R signalling pathway, or in the components of the IL-12 pathway, engenders susceptibility to intracellular infections, particularly tuberculosis, highlighting the critical role for this cytokine in host defence.

3.3 Cytokines 243 Th17 cells Th17 cells (ROR γ T) are a recently described subset implicated in autoimmunity and host defence. They release IL-17A, IL-17F, and IL-22, and when targeted in many rodent models of autoimmunity are found to be of profound pathologic importance. Their generation depends variously on the activities of IL-21, IL-1 β , IL-6, IL-23, and TGF β . IL-17A is a potent effector cytokine (20–30 kDa) that operates in synergy with IL-1 and TNF to promote leucocyte activation, bone marrow leucocyte maturation, haemopoiesis, and matrix degradation, the latter via direct effects on fibroblasts and chondrocytes. IL-22 similarly promotes autoimmune-mediated tissue damage by directly activating tissue cells such as keratinocytes and invading leucocytes. IL-17F is a homologue that exerts similar effector function but apparently with lower potency. Recently it has emerged that it may play a more important role in human immunity than previously thought from murine studies raising interesting in dual IL-17A/F inhibition. Th2 cells Th2 cells (GATA3) are characterized by IL-4, IL-5, IL-13, and IL-25 release, and have their primary role in driving humoral immunity and host defence to many parasites, particularly in mucosal/barrier defence. In disease states, Th2 cells promote especially allergy and anaphylaxis. Th2 differentiation is driven by IL-33 and IL-4. A further subset of regulatory T cells (Foxp3; Tr) is now described that comprises naturally occurring T cells that function in a predominantly suppressive manner via direct cell contact with adjacent leucocytes or via release of IL-10 and TGF β . Tr differentiation is favoured by TGF β and IL-35. Mechanisms that upregulate regulatory T-cell function (e.g. via use of CD28 superagonists or indeed of recombinant IL-35 itself, are now under investigation). Miscellaneous activities IL-6 is a pleiotropic proinflammatory cytokine that mediates function via IL-6R α and the common coreceptor, gp130. IL-6 also activates B cells, promoting isotype switching and immunoglobulin production and T cells promoting proliferation and differentiation. It has an important role in haemopoiesis and thrombopoiesis. IL-6 mediates intriguing systemic effects—it critically regulates the acute-phase response via direct effects on hepatocytes. Moreover, it has a role in integrating inflammatory responses with function of the hypothalamic pituitary adrenal axis indicative of a role in the immediate stress response. High levels of IL-6 expression are described in rheumatoid arthritis and Crohn's disease and in juvenile inflammatory arthritis, particularly systemic variants thereof, and Still's disease. High levels of IL-6 are also detected in Castleman's disease. IL-6 deficiency or blockade is anti-inflammatory in several in vivo and in vitro disease models, providing strong rationale for IL-6 blockade in the clinic. In contrast, IL-10 exhibits predominantly anti-inflammatory effects. It is released by a variety of leukocytes including macrophages, and T and B lymphocytes. Acting via IL-10RI and IL-10RII, it inhibits macrophage cytokine and RNI/ROI production, T-cell activation, dendritic cell priming and

maturation, and fibroblast activation. Note, however, that IL-10 promotes B-cell activation and immunoglobulin secretion. Several members of the IL-10 superfamily are now defined with effects often manifest in barrier defence. In particular, IL-20 and IL-22 are implicated in keratinocyte responses to cutaneous inflammation. The range of known cytokine activities described is now substantial across a variety of tissue compartments and processes. Often their original designation belies pleiotropic effects across a range of physiology and pathologies. Colony stimulating factors such as GM-CSF, G-CSF, and M-CSF were originally defined on the basis of leucocyte precursor differentiation and maturation, but now are recognized to play a role in effector immune responses. GM-CSF in particular is now attracting attention as a pivotal effector cytokine in the pathogenesis of some chronic inflammatory diseases—phase II trials are ongoing in rheumatoid arthritis using a GM-CSFR inhibitor. TGF β isoforms have broad effects in tissue maintenance and repair and enjoy broad expression and functional promiscuity. They initially promote inflammatory cell recruitment and activation together with a key role in fibroblast activation and matrix synthesis, but over time promote suppression of inflammation to permit efficient wound repair. The related cytokine family of bone morphogenetic proteins (BMP) exhibit similarly wide activities in tissue morphogenesis and repair. Comprising a large family of homo- and heterodimers, they regulate chemotaxis, mitosis, and differentiation during chondrogenesis, osteogenesis, and tissue morphogenesis in heart, skin, eye, and beyond, rendering them interesting moieties for therapeutic manipulation of tissue repair following injury, neoplasia, or inflammatory insult. Other fundamental processes in tissue repair also reside in cytokine regulation. Angiogenesis is critically regulated by vascular endothelial growth factor (VEGF) and by basic fibroblast growth factor (FGF). Together with naturally occurring inhibitors of angiogenesis, such as endostatin, this pathway is permissive for tissue repair, but also for maintenance of inflammation, or metastasis. These angiogenins carefully orchestrate recruitment of endothelial precursors and their subsequent maturation and organization into vessel structure—relative deficiency or excess can have significant consequences for tissues. Cytokines as therapeutic targets and biomarkers General concepts in cytokine therapeutics Cytokine immunology has provoked the creation of a dynamic new field in drug discovery. Cytokines represent therapeutic entities in themselves, best exemplified in their use to amplify cancer therapeutics via immune stimulation or to enhance immune competency in immunosuppressed patients. However, their short half-life and tendency to mediate systemic effects that are in general undesirable has limited their value in this respect. More success has been achieved in the treatment of chronic viral infection whereby treatment of hepatitis C infection with type I interferon as part of combination antiviral therapy leads to improved viral clearance. Type I interferons have also delivered benefits in the treatment of MS. Similarly, the utility of ‘anti-inflammatory’ cytokines as therapeutic entities per se in inflammatory diseases has been limited by their half-life and by toxicities arising from non-disease-related, but plausible, biological effector function. For example, IL-10, a cytokine with generally anti-inflammatory effects, exhibited some efficacy in patients with psoriasis and rheumatoid arthritis but offered an unacceptable toxicity/benefit ratio overall due in part to systemic adverse effects. Current efforts are focused upon delivering high

244 SECTION 3 Cell biology local concentrations of cytokine, perhaps by gene delivery methodologies, often in structurally modified form (e.g. by addition of an Fc domain). IL-2 is a promising emerging therapeutic with the potential to expand regulatory T cells and thus promote immune tolerance. Alternatively, tissue-localizing molecules (e.g. monoclonal antibodies against tissue endothelium specific targets, or damaged tissue epitopes) may be engineered on to cytokines at

the molecular level with the objective of providing high concentrations of local cytokine agonist activity in areas of maximal inflammatory insult. This remains an area under development. The crucial advance in recent years, however, has been the recognition that specific cytokine inhibition can bring about remarkable changes in inflammatory disease expression. Cytokines can be inhibited by interruption of any part of their synthesis, secretion, and effector pathway. Current therapeutics relies mainly on inhibition of cytokines in the extracellular or membrane compartment mediated by large biological drugs. Biological drugs generated thus far comprise antibodies and soluble cytokine receptor fusion proteins. Monoclonal antibodies specific for a given cytokine, which are either 'fully human' (human sequence; e.g. adalimumab, ustekinumab), 'humanized' (rodent sequence modified to resemble human structure), or chimaeric (part of the antibody is rodent derived and part of human structural origin, e.g. infliximab), offer effective high-affinity inhibition. Similarly, soluble cytokine receptors, fused with Fc domains of immunoglobulin to enhance their half-life and stability, similarly provide high-affinity therapeutic inhibitors (e.g. etanercept). Novel modifications to biologic agents, such as addition of polyethylene glycol residues (PEGylation), are in development to provide further refinement of their pharmacokinetic and pharmacodynamic properties. Other approaches to cytokine inhibition include the generation of small-molecule inhibitors that may target several points in the synthesis and release pathway:

- enzymes that cleave cytokines intracellularly, or from the cell membrane (e.g. TNF- α -converting enzyme (TACE), caspase 1)
- signal transduction molecules that mediate receptor intracellular function (e.g. syk, JAK, p38MAPK, JNK, NF- κ B)
- receptor antagonists selected for direct inhibition of cytokine receptor-protein interaction

An important corollary to small-molecule inhibition, at least for signal transduction inhibitors, is the loss of the exquisite specificity for a single cytokine contained in the biologicals. Thus, pathways such as the MAPK and JAK appear tractable to drug targeting, but since these molecules subserve several inflammatory cytokine receptor pathways, their inhibition is no longer specific to one pathway. The capacity to inhibit several cytokine effector pathways (and hence checkpoints) may be advantageous in bringing higher efficacy but must be carefully balanced with potential toxicity—predictable or idiosyncratic. At this time, the approach to inhibition of MAPK has failed in inflammatory illnesses: p38 inhibitors in particular have been trialled in numerous scenarios and as yet have offered no benefit. By contrast, JAK inhibitors are currently offering promise. Tofacitinib is a JAK1/JAK3 inhibitor approved for use in some geographic areas in the treatment of rheumatoid arthritis. Follow on compounds that inhibit JAK1/JAK2 (baricitinib) and JAK1 (upadacitinib, filgotinib) are either now approved or will be so shortly. The critical safety efficacy window for such agents remains uncertain. Future approaches to cytokine inhibition will likely entail gene silencing via delivery of inhibitory RNA species, or gene therapeutic delivery of inhibitory biologic agents. Cytokine targeting in inflammatory diseases

Cytokine inhibitors are now widely used in several chronic inflammatory diseases including rheumatoid arthritis, inflammatory bowel disease, sarcoidosis, psoriasis, psoriatic arthritis, uveitis, and vasculitis and are under investigation in many more conditions. They are dealt with in detail in disease-related chapters. Whereas we have clearly used such therapeutics to inform the pathogenesis of diseases, we have also learned critical lessons from the use of these agents about the basic biology of cytokines:

- First, it appears that cytokines exist in highly regulated networks and that there are critical checkpoints in such networks at which inhibition leads to general suppression of the cascade.
- Second, there is redundancy in the cytokine system such that single cytokine blockade, although of therapeutic utility, does not lead to paralysis of the host-defence capability. That said, there are some defence processes that may heavily rely on one cytokine—thus TNF appears critical for granuloma formation, hence susceptibility to tuberculosis and some

fungal infections in TNF-blocked patients. • Third, from pharmacokinetic (PK) studies we can deduce that cytokine effects in tissues are quantitatively regulated and thresholds may exist for some of their effector function. • Fourth, we have not yet established sufficient rationale or understanding of cytokine networks to the level that we can formally combine targeting approaches. Combination targeting in rodent models is highly effective in many disease models, whereas in humans combined biological therapies have thus far led to increased toxicity with no yield in improved efficacy. Measuring cytokines in biological fluids There is increasing interest in measurement of cytokines in serum and plasma and ex vivo in cellular supernatants. This is in part driven by basic discovery research. However, there is intense interest in the potential for cytokines as biomarkers of disease activity and prognosis or for pharmacodynamic purposes to predict drug response or toxicities. Cytokines were originally measured and defined in bioassays using live cell assays in vitro that are impractical for such purposes. Thereafter, enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay became the techniques of choice. Though these are validated and still widely used, the future analysis of cytokines will be facilitated by the use of multiplex technologies, based on laser analysis, which measure up to 30 cytokines in small (<50 µl) volumes. Techniques based on protein chips offer further utility to measure the expression of wider ranges (up to 360 proteins per assay) of cytokines. The critical advance here will be to allow analysis of changes in patterns of cytokines as opposed to changes in single moieties that will in turn considerably increase the power of this approach. Moreover, such techniques can be adapted to evaluate expression not only in fluid phase but also in tissue extracts to allow evaluation in biopsies.

3.3 Cytokines 245 FURTHER READING Altan-Bonnet G, Mukherjee R (2019). Cytokine-mediated communication: a quantitative appraisal of immune complexity. *Nat Rev Immunology*, 19, 205–17. Dayer JM (2004). The process of identifying and understanding cytokines: from basic studies to treating rheumatic diseases. *Best Pract Res Clin Rheumatol*, 18, 31–45. Dinarello CA (2005). Blocking IL-1 in systemic inflammation. *J Exp Med*, 201, 1355–9. Dong C (2008). TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat Rev Immunol*, 8, 337–48. Elliott MJ, et al. (1994). Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet*, 344, 1105–10. Feldmann M, et al. (2004). The transfer of a laboratory based hypothesis to a clinically useful therapy: the development of anti-TNF therapy of rheumatoid arthritis. *Best Pract Res Clin Rheumatol*, 18, 59–80. Garlanda C, Dinarello CA, Mantovani A (2013). The interleukin 1 family: back to the future *Immunity*, 39, 1003. Jones SA, Jenkins BJ (2018). Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. *Nat Rev Immunology*, 18, 773–89. McInnes IB, Schett G (2007). Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol*, 7, 429–42. McNab F, et al. (2015). Type I interferons in infectious disease *Nat Rev Immunol*, 15, 87. Moreland LW, et al. (1997). Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N Engl J Med*, 337, 141–7. Ouyang W, Kolls JK, Zheng Y (2008). The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity*, 28, 454–67. Van den Berg W, McInnes IB (2013). Th17 cells and IL-17A—focus on immunopathogenesis and immunotherapeutics. *Semin Arthritis Rheum*, 43, 158.