5 Cytokines Are a Therapeutic Target for the Prevention of Inflammation-Induced Cancers

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Abstract

Interleukin-6 (IL-6) is an inflammatory cytokine with a well-documented role in cancer. The cytokine binds to a membrane-bound IL-6 receptor (IL-6R) and this complex associates with two molecules of the signal transducing protein gp130, initiating intracellular signaling. Whereas gp130 is expressed on all cells of the body, the IL-6R is only found on some cells, mainly hepatocytes and several leukocytes. Cells, which only express gp130 and no IL-6R, cannot respond to IL-6. We have shown that the IL-6R exists as a soluble protein generated by limited proteolysis of the membrane-bound receptor or by translation from an alternatively spliced mRNA. The complex of soluble IL-6R (sIL-6R) and IL-6 can bind to gp130 on cells that lack the membrane-bound IL-6R and trigger gp130 signaling. We have named this process trans-signaling. We review data that show that IL-6 uses classical signaling via the membrane-bound receptor and trans-signaling via the soluble receptor in physiological and pathophysiological situations. We have developed designer cytokines, which specifically enhance or inhibit IL-6 trans-signaling. These designer cytokines have been shown to be extremely useful in therapeutic applications such as blockade of chronic inflammation and cancer.

Cytokines

Cytokines are small soluble proteins, which are secreted by and act on a variety of different cell types. Because of structural features, cytokines can be grouped into different families. One of them, named after the first discovered member of the family is the interleukin 6 (IL-6) family (Taga and Kishimoto 1997). This family further comprises IL-11, ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin like cytokine (CLC), leukemia inhibitory factor (LIF), neuropoietin (NPN) and oncostatin M (OSM). Recently two new cytokines also belonging to the IL-6 family, named IL-27 and IL-31, have been found (Dillon et al. 2004; Pflanz et al. 2004). Each of the cytokines of the IL-6 family seem to have specific activities in different compartments but interestingly, in some cases the cytokines of the family can substitute each other. This phenomenon has been called redundancy.

Recent advances have documented a series of IL-6 activities that are critical for resolving innate immunity and promoting acquired immunity (Jones 2005; Jones et al. 2005). The transition between innate and acquired immunity is a central event in the resolution of any inflammatory condition, and disruption of this immunological switch may potentially distort the immune response and affect the onset of autoimmune or chronic inflammatory disorders (Hoebe et al. 2004).

Receptor Complexes

The structural basis of redundancy in the IL-6 family is the composition of the receptor complexes, via which the members of the IL-6 family transduce their signals into the target cells. The central protein of the receptor complexes is the

ubiquitously expressed glycoprotein gp130 (Taga and Kishimoto 1997). All receptor complexes use this protein as a signal transducing receptor subunit. Besides gp130, the receptor complexes may contain different ligand-binding proteins, which are specific for one cytokine, for example the receptors for IL-6, CNTF, and IL-11. Moreover, the receptor complexes for CNTF, CLC, CT-1, LIF, NPN, and OSM contain the LIF-receptor (LIF-R) protein, a signal-transducing receptor subunit which is homologous to gp130 (Taga and Kishimoto 1997).

Soluble Receptor Paradigm

When making contact with a target cell, IL-6 first binds to the specific IL-6 receptor (IL-6R). In a second step, this complex associates with two molecules of gp130, thereby initiating intracellular signaling (Taga and Kishimoto 1997; Rose-John 2001). Whereas gp130 is expressed on most if not all cells of the body, the expression of the IL-6R is restricted to hepatocytes, neutrophils, monocytes/macrophages, and some lymphocytes. Interestingly, cells that do not express the IL-6R on the cell surface can respond to IL-6 if a soluble form of the IL-6R (sIL-6R) is present (Mackiewicz et al. 1992; Taga et al. 1989) (Fig. 1a). Such a soluble IL-6R has been found in various body fluids. It turned out that the sIL-6R can be generated by two different mechanisms: limited proteolysis of the membrane protein and translation from an alternatively spliced mRNA (Lust et al. 1992; Rose-John and Heinrich 1994; Müllberg et al. 2000; Althoff et al. 2000, 2001; Hundhausen et al. 2003; Matthews et al. 2003; Abel et al. 2004). The stimulation of cells by the complex of IL-6 and sIL-6R has been termed trans-signaling (Rose-John and Heinrich 1994; Jones 2005; Jones et al. 2005). Because they lack a functional IL-6R on their surface, early hematopoietic progenitor cells (Peters et al. 1997, 1998; Audet et al. 2001; Hacker et al. 2003; Campard et al. 2006), embryonic stem cells (Rose-John 2002; Humphrey et al. 2004), endothelial cells (Romano et al. 1997), mesothelial cells (Hurst et al. 2001; McLoughlin et al. 2003), neural cells (März et al. 1998, 1999), smooth muscle cells (Klouche et al. 1999), and T cells (Atreya et al. 2000; Becker et al. 2004, 2005) only respond to IL-6 in the presence of the sIL-6R.

Designer Cytokines

Construction of chimeric proteins containing modules of different proteins belonging to the IL-6R family helped us to identify cytokine binding motives on receptor proteins (Kallen et al. 1999 2000; Aasland et al. 2002, 2003). On the basis of these experiments together with other structural information available on membrane bound and soluble cytokine receptors, we designed several cytokine–cytokine receptor molecules.

Covalent linkage of the domains of the IL-6 and sIL-6R that are necessary for biological activity via a flexible polypeptide linker resulted in a recombinant fusion protein, that was found to be 100–1,000 times more active than the native IL-6/sIL-6R complex. Because of this enhanced activity, we termed this cytokine Hyper-IL-6 (Fischer et al. 1997). Cells lacking the IL-6R, which as a consequence do not respond to IL-6 alone, such as embryonic stem cells, endothelial cells, hematopoietic progenitor cells, neuronal cells, and smooth muscle cells, show a remarkable biologic response to Hyper-IL-6. Adoption of this approach led to the construction of fusion proteins between IL-11 and the soluble IL-11R (Pflanz et al. 1999) Furthermore, proteins consisting of CNTF fused to the soluble CNTF-R have been constructed. These proteins show high neurotrophic activity on neural cells lacking a surface CNTF-R (Guillet et al. 2002; Sun et al. 2002).

By fusing the entire extracellular domain of gp130 protein to the Fc region of human IgG1, we constructed a soluble form of gp130 (sgp130Fc). Surprisingly, in HepG2 cells this protein inhibited only the production of the acute-phase protein antichymotrypsin (ACT) induced by Hyper-IL-6, whereas the response to IL-6 alone remained unaffected (Jostock et al. 2001). We concluded that sgp130Fc does not interfere with responses mediated by the membrane bound IL-6R but exclusively inhibits IL-6 trans-signaling via the soluble IL-6R (Fig. 1b).

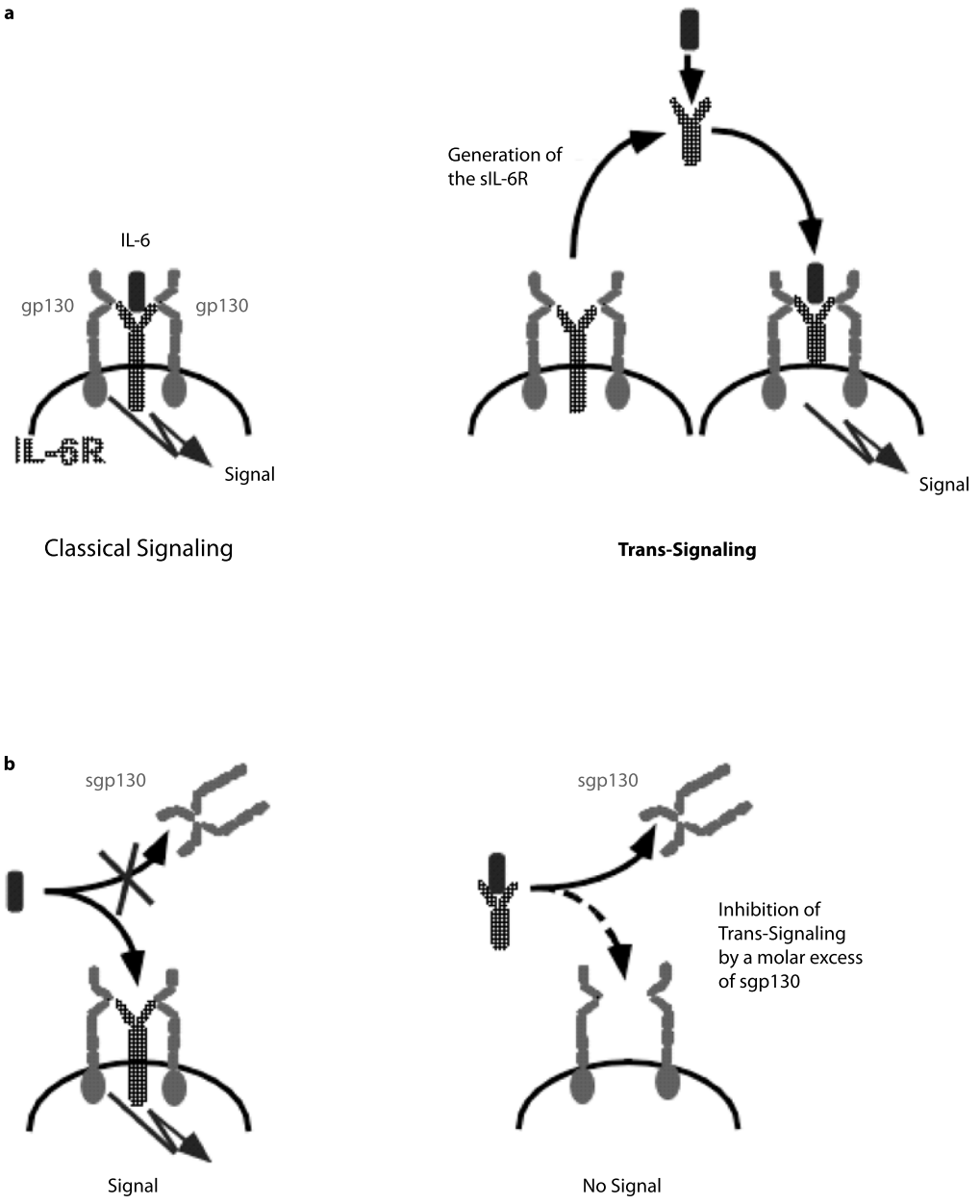

Fig. 1a, b Classical and trans-signaling of IL-6 and the inhibitory mechanism of sgp130. **a** The two modes of IL-6 activation: classical IL-6 activation via the membrane-bound IL-6R and trans-signaling via a soluble form of the IL-6R. In both cases, signals are transmitted by membrane-bound gp130. **b** Sgp130 binds the IL-6/sIL-6R complex to antagonize IL-6 trans-signaling, whereas classical signaling remains unaffected

Since gp130 is part of the receptors of the cytokines IL-6, IL-11, IL-27, CLC, CNTF, CT-1, LIF, OSM, and NPN, we asked whether the sgp130 inhibition was specific for the IL-6/sIL-6R complex or whether sgp130Fc also effected the biologic activity of the other IL-6-family cytokines (Jostock et al. 2001; Scheller et al. 2005). Our experiments showed that CNTF-mediated activities were unaffected by the sgp130Fc protein. To inhibit the proliferative activity of LIF and OSM on BAF/3 cells stably transfected with gp130 and LIF-R cDNAs, more than 100-fold higher sgp130Fc concentrations were needed than for the inhibition of Hyper-IL-6. These findings are in agreement with surface plasmon resonance experiments showing that Hyper-IL-6 and OSM bind to sgp130 with KD values of 6.9×10^{-9} M and 1.6×10^{-7} M, respectively (Richards et al. 2006). Because the recently found cytokine IL-27 also acts via a receptor complex consisting of gp130 and the related receptor protein WSX-1 (Pflanz et al. 2004), we examined whether sgp130Fc inhibited the biologic activity of IL-27 (Scheller et al. 2005). Our results clearly indicated that the sgp130Fc protein did not affect the IL-27-mediated STAT3 phosphorylation and proliferation of BAF/3 cells expressing gp130 and WSX-1. As a conclusion of these results, we postulated that the sgp130Fc protein is a specific inhibitor of the IL-6/sIL-6R trans-signaling complex.

Physiological Role of Soluble gp130

In experimental arthritis, colitis, and colon cancer, sgp130 has been shown to influence leukocyte trafficking and to reduce the severity of the diseases. Moreover, the phenotype of sgp130 transgenic mice resembles the phenotype of mice treated with sgp130 in vivo. Together these findings led us to the conclusion that sgp130 acts as the natural inhibitor only of IL-6/sIL-6R complexes. IL-6 does not directly bind sgp130, and as a consequence the classical IL-6 signal via the membrane forms of the IL-6R and gp130 remains unaffected. Figure 1b shows our concept of the molecular mechanism, by which soluble gp130 exerts selective inhibition of the IL-6/sIL-6R complex. The IL-6/sIL-6R complex shows equal affinity for the soluble and membrane-bound variants of sgp130, and a molar excess of sgp130 inhibits IL-6 trans-signaling. The selective inhibition of IL-6 trans-signaling appears to be an important pathophysiological regulation mechanism in inflammatory diseases.

IL-6 Trans-signaling and Inflammation

Many investigations have shown that IL-6 plays an important role in the transition from innate immunity to acquired immunity, a crucial event in the controlling of any inflammatory states (Hurst et al. 2001; Jones 2005; Jones et al. 2005). Disruption of this essential switch leads to an inappropriate immune response and might cause the onset of autoimmune or chronic inflammatory disorders (Hoebe et al. 2004). The discovery of the IL-6 trans-signaling mechanism may help to understand the contradictory role of IL-6 in acute and chronic inflammatory states (Kallen 2002). In acute inflammation such as septic shock (Ulich et al. 1991; Barton and Jackson 1993; Diao and Kohanawa 2005), IL-6 shows favorable effects, whereas under chronic inflammatory conditions, IL-6 seems to maintain the disease state. The contribution of IL-6 trans-signaling and the interference of IL-6 signaling with STAT1, IFN-γ, TGF-β, GATA-3, and NF-κB remarkably influence disease outcome (Becker et al. 2004; Doganci et al. 2005; Hegde et al. 2004; McLoughlin et al. 2005). Studies have been conducted to examine the role of IL-6 trans-signaling in acute inflammation, asthma, tumor expansion, and the inflammatory response associated with tumor progression. The common role of IL-6 trans-signaling in these various disease states was an orchestration of leukocyte recruitment and activation and control of apoptotic processes (Jones 2005; Jones et al. 2005). In this context, IL-6 suppresses neutrophil infiltration while promoting attraction and activation of mononuclear leukocytes, leading to a switch from the innate to the acquired immune system (Atreya et al. 2000; Becker et al. 2004; Hurst et al. 2001; McLoughlin et al. 2005). Interrupting IL-6 trans-signaling in experimental models of colitis and rheumatoid arthritis improved disease outcome (Atreya 2000; Nowell et al. 2003). This effect is caused by either inhibiting the recruitment or increas-

ing the apoptotic clearance of mononuclear cells. Both effects lead to a diminished mononuclear cell concentration which are at least partly responsible for the chronic disease progression in inflamed tissue. The observation that IL-6 trans-signaling is a contributor to chronic disease progression is underlined by several in vitro and in vivo studies in which IL-6 inhibited apoptosis by inducing anti-apoptotic regulators via the STAT3 signal transduction pathway (Atreya et al. 2000; Teague et al. 2000; Curnow et al. 2004). The understanding of IL-6 mediated control of activated mononuclear cell populations is therefore a hallmark in understanding of chronic disease progression (Jones 2005; Jones et al. 2005).

Results from studies with IL-6 knockout mice show that IL-6 influences T cell recruitment via the local secretion of chemokines (CXCL10, CCL2, CCL4, CCL5, CCL11, CCL17) and the expression of chemokine receptors (CCR3, CCR4, CCR5, CXCR3) on CD3+ cells (Hurst et al. 2001; McLoughlin et al. 2005). Since sgp130 selectively antagonizes IL-6 trans-signaling in vivo without affecting the classical pathway via the membrane receptor, it is possible to distinguish between both mechanisms. Interestingly, sgp130 only blocked chemokine expression, whereas T cell chemokine receptor expression remained unaffected (McLoughlin et al. 2005). Differential activities for IL-6 classical and trans-signaling were also defined using a murine asthma model. In this case, IL-6 alone directed the T cell population toward the Th-2 subtype, whereas for the activation of this Th2 population IL-6 trans-signaling is necessary (Doganci et al. 2005).

In summary, T cell responses are differentially regulated by IL-6 alone and by the IL-6/sIL-6R complex. In this context, it is interesting to address the question whether T cells universally express IL-6R protein on their surface, or whether the expression of IL-6R is restricted to specific subsets of T cells. Of CD3+ T-cells from the circulation, 35%–45% express IL-6R, whereas only 2%–5% of CD3+ cells infiltrating an inflammatory tissue are IL-6R-positive (Atreya et al. 2000; Becker et al. 2004; Curnow et al. 2004). Thus under inflammatory conditions, the expression of the IL-6R or the homing of a CD3+IL-6R– T cell subset to inflammatory foci is dramatically downregulated. T cells treated in vivo with superantigen also show significantly lower levels of IL-6R (Teague et al. 2000). Thus, the loss of the IL-6R on the surface of T cells might be a marker for a less activated status of these cells.

Furthermore, IL-6-mediated control of cellular differentiation also takes place in the polarization of monocytic cells. The major observed effect is the inhibition of differentiation of monocytic cells toward dendritic cells, thus favoring the development of a macrophage phenotype (Chomarat et al. 2000; Mitani et al. 2000). The shifting of the differentiation of human monocytes can also be seen in IL-6–/– mice in vivo, where the expansion of bone marrow cells resulted in a tenfold higher number of CD11c+ dendritic cells as compared with wild type mice (Bleier et al. 2004). Interestingly, the activity of dendritic cells in IL-6–/– mice is impaired, implicating that IL-6 is also necessary for their activity. In this context, IL-6 has been shown to inhibit NF-κB activity and suppress the expression of the chemokine receptor CCR7 in dendritic cells (Hegde et al. 2004). Furthermore, IL-6 secretion by dendritic cells following toll-like receptor activation blocks the immunosuppressive activities of regulatory T cells (Pasare and Medzhitov 2003). Thus IL-6 not only seems to be responsible for the recruitment of dendritic cells, but also modulates the activity of dendritic cells in advancing adaptive immune reactions.

In summary, these studies highlight the central role of IL-6 in both innate and acquired immune responses. Changes in IL-6 production and in the expression of IL-6R on the surface of target cells may support the development of chronic inflammatory diseases. The finding that the IL-6 trans-signaling pathway has specific functions in chronic disease progression and the fact that a selective inhibitor for this pathway is available opens new promising perspectives for therapeutic intervention.

Colon Cancer as an Example of Inflammatory Induced Cancer

In earlier studies, we demonstrated the importance of the IL-6 trans-signaling pathway for the maintenance of chronic inflammatory bowl disease (Crohn disease) (Atreya et al. 2000).

Interestingly, our studies showed that in Crohn disease patients the T cells from gastric tissue are extremely resistant to apoptosis. Biochemical examinations showed that these T cells produced large amounts of IL-6 and that the intracellular JAK-STAT signal transduction pathway was activated. Surprisingly, treatment of these cells with a neutralizing monoclonal antibody to IL-6R induced apoptosis, although the cells lacked a membrane form of the IL-6R. As the treatment of the cells with sgp130 also induced apoptosis, this effect must have been mediated via the IL-6 trans-signaling pathway (Fig. 2). This finding showed that IL-6 trans-signaling is responsible for the surveillance of activated T cells within gastric tissue from Crohn disease patients by inhibiting apoptotic processes. The sIL-6R needed for IL-6 trans-signaling is most likely provided by shedding of the membrane IL-6R from lamina propria macrophages or infiltrating neutrophils (Atreya et al. 2000; Jostock et al. 2001). Interestingly, it was recently found that in addition to the levels of IL-6, the levels of sIL-6R and sgp130 are also elevated in chronic inflammatory bowel diseases, and that the IL-6 found in the circulation was preferentially complexed to sIL-6R and sgp130 (Mitsuyama et al. 2005).

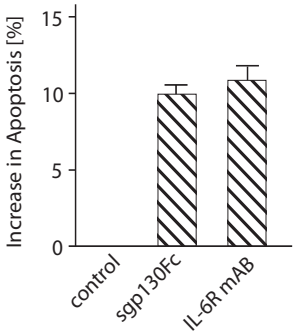

Fig. 2 Apoptosis of lamina propria mononuclear cells (LPMC) of Crohn disease patients upon treatment with sgp130. LPMCs were isolated and cultured for 48 h in the presence or absence of 10 μg/ml of a neutralizing mAB-specific for human IL-6R or 10 μg/ml sgp130Fc. Cells were stained for annexin V and propidiumiodide and analyzed by FACS. The increase in apoptotic (annexin V-positive and propidiumiodide-negative) cells is shown. The data presented are means of triplicate measurements with standard errors shown as vertical bars

Moreover, the production of sIL-6R as well as IL-6 trans-signaling processes play a dominant role in tumor cell growth. Interestingly, cross-talk between the TGFβ and IL-6 pathway was demonstrated (Fig. 3). Inhibition of TGFβ production resulted in an increased IL-6 production in mice. Surprisingly, the IL-6 overproduction is accompanied by a loss of membrane bound IL-6R from the cell surface of epithelial cells within tumor lesions. This loss of membrane bound IL-6R was most likely due to an increase of the cell surface expression of the protease ADAM17, which is responsible for cleavage of the IL-6R (Matthews et al. 2003). As the epithelial tumor growth could be inhibited either by a neutralizing antibody directed against the IL-6R or by sgp130Fc, we concluded that the growth of the tumor was promoted by IL-6 trans-signaling but not by the classic signaling via the membrane-bound IL-6R (Becker et al. 2004, 2005). The same observations of downregulation of the IL-6R on the surface of tumor epithelial cells and the upregulation of ADAM17 were made in human colon cancer patients, implying that a similar mechanism operates in human and mouse colon cancer development (Becker et al. 2005). These findings show that interrupting IL-6 trans-signaling with sgp130Fc in colon cancer patients will be a promising new therapeutical strategy.

Conclusions

The alternative signaling pathway of IL-6 via the sIL-6R/IL-6 complex seem to be an important mechanism for the development of chronic inflammatory diseases and inflammation-associated tumor growth. The ability of selective inhibition of sIL-6R-dependent IL-6 responses with sgp130 can be used in vivo to distinguish between classical IL-6 signaling and IL-6 trans-signaling (Fig. 4). There is now clear evidence that chronic inflammatory states often lead to neoplastic lesions. Sgp130 can be used to effectively block immunological processes that promote inflammatory disease progression. One clear advantage of selectively blocking IL-6 trans-signaling is the fact that classic IL-6 responses remain unaffected. These include the hepatic acute-phase response, which plays an important role in the defense of the body against infections and trauma. These

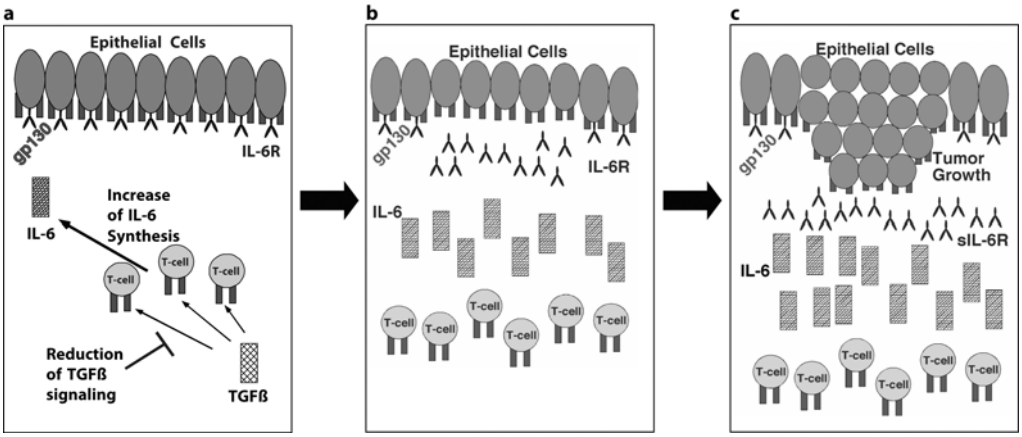

Fig. 3a–c Schematic model of TGF-β-regulated IL-6 trans-signaling in experimental colon cancer. **a** A reduction of TGFβ signaling results in increased IL-6 secretion. **b** The observed upregulation of the ADAM17 metalloproteinase on epithelial cells results in shedding of the IL-6R. Upon stimulation with the IL-6/sIL-6R complex, T cells become resistant to apoptosis and therefore the number of T cells increases. **c** Growth of epithelial cells in tumor lesions is observed upon stimulation with the IL-6/sIL-6R complex. Tumor growth can be inhibited by blocking the IL-6R using a monoclonal antibody or by inhibiting IL-6 trans-signaling with the sgp130Fc protein. Dark grey symbols, gp130; black symbols, membraned bound IL-6R and soluble IL-6R; light grey symbols, epithelial cells

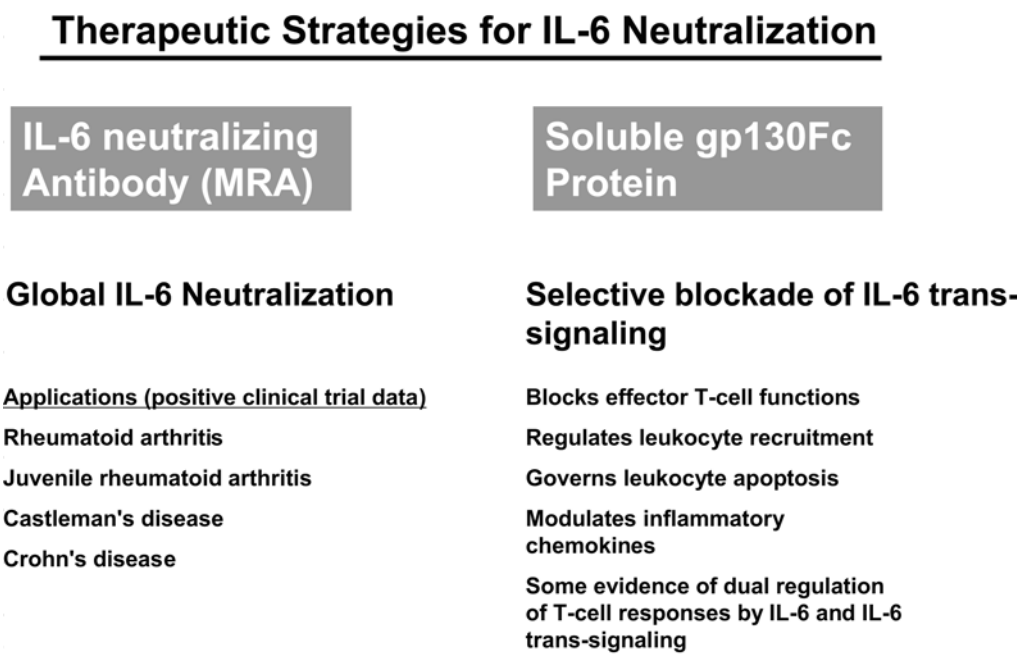

Fig. 4 Therapeutic strategies for targeting IL-6 signaling. Current therapeutic regimes designed to clinically suppress IL-6 activities involve the application of the blocking monoclonal antibody MRA (atlizumab, tocilizumab) effective against a range of conditions. This did not distinguish between classical IL-6 signaling and IL-6 trans-signaling. The fusion protein sgp130Fc selectively targets IL-6 trans-signaling, and leaves classical IL-6 signaling intact

considerations have provided a therapeutic rationale for the administration of sgp130 in a series of chronic inflammatory conditions, which are prone to progress to cancer.

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