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Popular Article

Novel strategies to modulate cytokine action for therapy

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Cytokines are secreted or membrane presented, Signaling and immunoregulatory molecules that mediate broad cellular functions, including development, differentiation, growth, and survival. Accordingly, the regulation of cytokine activity is extraordinarily important both physiologically and pathologically. Various physiological functions of cytokines were described below (1).

Cytokines are secreted by variety of cell types (Fig 1). Based on the structure and function, cytokines are broadly classified into six families as depicted in Fig-2.

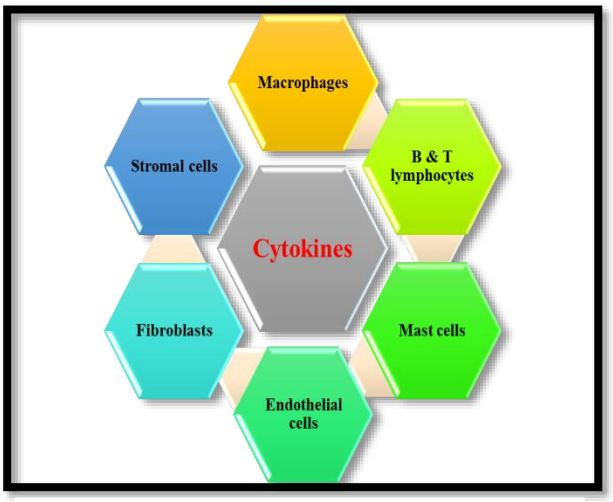

Fig 1: Cytokine secreting cells

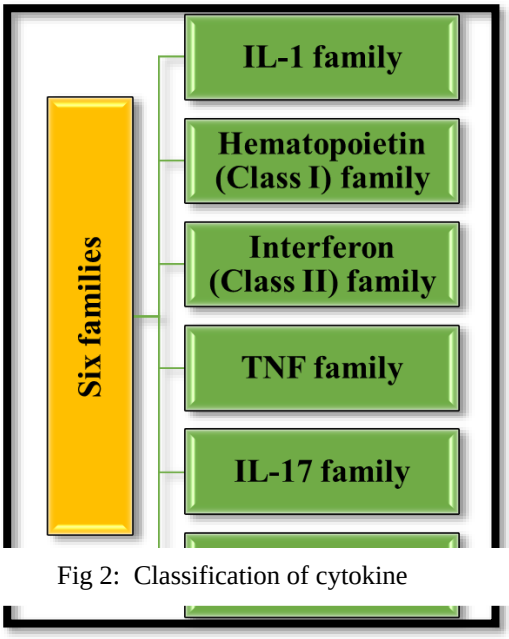

Fig 2: Classification of cytokine

Classification of cytokine receptors:

1. **Type I cytokine receptors:** The extracellular amino acid domain of these receptors contain conserved motifs, Fibronectin III and WSXWF motif.
2. **Type II cytokine receptors:** Structurally these receptors resemble type I receptors except a WSXWF motif.
3. **Immunoglobulin (Ig) receptors:** These receptors are structurally similar with immunoglobulins and distributed ubiquitously throughout various cells and tissues of the vertebrate body.

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4. **Tumour necrosis factor (TNF) receptors:** These receptors have a cysteine-rich common extracellular binding domain (2).
5. **Chemokine receptors:** They are G-protein coupled receptors acting as binding proteins for HIV (CXCR4 and CCR5).
6. **IL-17 receptors:** IL-17 receptors contain the longest cytoplasmic tail with docking sites for numerous signalling intermediates.

Characteristics of Cytokines

1. Cytokines are different from hormones, their action restricted to specific area.
2. Cytokines are produced by a broad range of cells.
3. Cytokines act through mainly cell surface receptors.
4. Cytokines show pleotropic property, i.e., one cytokine may have many different target sites and its action depends on the type of target site.
5. Many cytokines produce the same action (redundancy) acting on the same type of receptor. Some produce synergistic and some promote antagonistic interaction among themselves (3).

Signal transduction mechanisms of cytokines

Most of the cytokines exert their action through JAK-STAT pathway. Cytokine receptors are tyrosine kinase linked receptors without enzymatic activity in its cytoplasmic domain. Dimerization is initiated by ligand (cytokine) binding, followed by phosphorylation of JAK proteins. Then JAKs recruit and phosphorylate other proteins such as STATs (Signal transducers and activators of transcription) which contain SH_2 groups, and this prompts dimerization and translocations of STATS into to nucleus. Finally, STATS bind to DNA and transcription of specific target gene takes place. There are 4 members in the JAK family: JAK1, JAK2, JAK3, and TYK2. The STAT family comprises of seven members: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 (4).

Apart from JAK-STAT receptor pathway some of cytokines exert their effects through pathways like Mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt). Signaling mechanism is very much crucial to augment or inhibit the cytokine action (1).

Some proteins which negatively regulate the cytokine action, for example cytokine inducible SH_2 protein (CIS) and suppressor of cytokine signalling (SOCS)1 and 3 have an amino-terminal kinase-inhibitory region (KIR) that inhibits JAK tyrosine kinase activity and a carboxy-terminal SOCS-box that recruits the ubiquitin-transferase complex. Growth hormone, EPO and other cytokines stimulate the expression of these proteins (5).

The key functions of cytokines are:

1. Development of cellular and humoral immune responses
2. Induction of the inflammatory response
3. Regulation of haematopoiesis
4. Control of cellular proliferation and differentiation
5. Healing of wounds (tissue damage repair)
6. Control of cell replication and apoptosis
7. Anti-tumour activity

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The major challenges in cytokine therapy include-

- Inaccurate targeting due to pleiotropic and redundancy property.
- Any alteration in cytokine action leads to impaired immune response in the body.
- The isolation and production of cytokines require sterile conditions and multiple stages of purification.
- Cytokines have limited shelf life and require special/ controlled storage conditions.

Fig 3: Therapeutic challenges of cytokines

Even though cytokines have a wide range of therapeutic activities, clinical applications are limited due to above mentioned therapeutic challenges (Fig. 3). To address these issues, specific strategies for modulating cytokine activity are being developed.

Various approaches to therapeutically enhance cytokine activity

1. Prolonging cytokine half-life by protein fusions and PEGylation

A short in vivo half-life and toxicity of natural cytokines are two characteristics that may restrict their delivery (6). There are several methods for extending the half-life, including fusing to other proteins (such as albumin and immunoglobulin) (7).

PEGylation, which involves the addition of PEG polymers to proteins to lengthen their half-life in vivo, has been used for years to prevent immunogenicity and defend against protein breakdown (8).

2. Optimizing the delivery of cytokines

The delivery of cytokines can be influenced using a variety of technical strategies. The main components of these include polymeric matrices, microparticles such as polymeric microparticles, and nanoparticles. However, studies are lacking with these cytokine delivery techniques (9).

In the tumour microenvironment (TME), cytokines can also be selectively activated. As an instance, WTX-124 (Indukine), an IL-2 pro-drug, is activated after being broken down by certain proteases in the TME (10).

3. Immunocomplexes and immunocytokines

Creating an immunocomplex is an additional method of extending a cytokine's half-life and potentially biasing its receptor interaction with domain. For example, S4B6 mAb (monoclonal antibody) supports IL-2Rβ interaction by blocking IL-2α contact with IL-2R, which encourages the proliferation of cells with low IL-2Rα expression and T_{eff} cell expansion. In contrast, JES6-1 sterically blocks the ability of IL-2 to interact with IL-2Rβ, promoting expansion of cells expressing IL-2Rα (particularly Treg cells) (11).

4. Engineering cytokine receptors or cytokine–receptor pairs

Orthogonal cytokines are created altering the cytokine and extracellular domain of the receptor

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but retaining the cytoplasmic domain and thus the signal. Based on cytokine families that share common chains, cytokines likely evolve in concert with distinctive receptor chains (12).

5. Cytokine muteins

The creation of cytokine muteins is a technique that has been utilised for many years to produce molecules with changed activity, either increased or lessened or qualitatively altered, where mutagenesis can possibly boost or lower the affinity of a cytokine. When cytokines bind with many cytokine receptor chains, each contact can be selectively targeted and may have potentially differential effects (1).

6. Neokines

An innovative strategy for producing new molecules of potential therapeutic value is the de novo computationally generated proteins that imitate the binding sites of natural cytokines but have a more compact structure and show little resemblance to the native cytokines. For example, neo-IL-2/IL-15 (NL-201) has a topological structure that is considerably different from that of IL-2 or IL-15. With improved packing, NL-201's up-down-up-down structure still maintains the important surface interactions with IL-2Rβ and γ chains (13).

Various approaches to therapeutically suppress cytokine activity

1. Antibodies to cytokines and cytokine receptors

Several unique cytokine-blocking mAbs have been developed. Tocilizumab, one of them, targets the IL-6 receptor and is a treatment for cytokine release syndrome, rheumatoid arthritis, juvenile rheumatoid arthritis, and Castleman disease, in which IL-6 is overproduced (14).

2. JAKs and STATs antagonists

Tofacitinib was developed as a JAK3 preferably selective inhibitor, with less potent effects on JAK1 and JAK2. Tofacitinib blocks the Signaling of all six γc-family cytokines. Tofacitinib, which was first licenced for the treatment of rheumatoid arthritis, now has a variety of applications. In principle, selective STAT inhibition may be more specific than JAK inhibition (1).

Targeted protein degradation

The PROTAC (proteolysis-targeting chimeras) molecules are bifunctional, with one end binding to a protein of interest (POI) and the other end binding to an E3 ligase to form a ternary complex, in which the E3 ligase then affects the transfer of ubiquitin from an E2 enzyme to the POI, marking it for proteasomal degradation. Another approach to target degradation of extracellular and cell surface proteins uses a lysosomal degradation strategy, termed KineTACs for cytokine receptor targeting chimeras (15&16).

Conclusion

Cytokines are signalling molecules that play critical roles in a variety of biological processes

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that help to maintain homeostasis. Understanding the structure of cytokines and cytokine receptors, receptor biology, and intracellular signalling processes is essential for their therapeutic regulation. Cytokine deficiencies or storm may be readily regulated by replenishment or blocking, respectively, but overcoming the phenomena of cytokine pleiotropy is the key issue. Another significant area is the capacity to fine-tune the effects of cytokines; however, progress has been limited so far.

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