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COMMENTARY

Directional migration of leukocytes: their pathological roles in inflammation and strategies for development of anti-inflammatory therapies

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ABSTRACT

Directional migration of leukocytes is indispensable to innate immunity for host defense. However, recruitment of leukocytes to a site of tissue injury also constitutes a leading cause for inflammatory responses. Mechanistically, it involves a cascade of cellular events precisely regulated by temporal and spatial presentation of a repertoire of molecules in the migrating leukocytes and their surroundings (microenvironments). Here I will summarize the emerging evidence that has shed lights on the underlying molecular mechanism for directional migration of leukocytes, which has guided the therapeutical development for innovative anti-inflammatory medicines.

Key words: *Directional cell migration, leukocytes, inflammation, therapy.*

INTRODUCTION

Directional cell migration is a basic feature of many cell types in a variety of species. It has been known for centuries. For example, migration of immunological cells has been described in the 1800s [1]. Since then, the biological importance of directional cell migrations has been recognized and emphasized. It participates in various aspects of biological development, such as fertilization, embryogenesis, patterning, tissue and organ formation. It also involves in various physiological and pathological processes, including homeostasis, lymphocyte homing, leukocyte trafficking and recruitment, thrombosis, angiogenesis, wound healing and cancer metastasis[2],[3].

During the past twenty years, a variety of molecules participating in distinct aspects of mammalian cell migration have been discovered and functionally characterized. The molecular mechanisms governing the precise regulations of these cellular events, such as temporal and spatial expression of cell adhesion molecules, gradient formation of chemokines and chemotactic factors, proteanases, signal transduction molecules, cytoskeletal rearrangements and so on, have begun to emerge[2-19]. In this review, I will focus on the general pictures of molecular mechanisms governing the directional migration of leukocytes and the application of this knowledge in the research and development of anti-inflammatory medicines.

Leukocytes functioning as a double edged "sword"

Recruitment of leukocytes to the site of bacterial and viral infection is integral to innate immunity. The recruited leukocytes are known to engulf (phagocytosis), kill (microbicidal activity; for example, release superoxide, hydrogen peroxide and hydroxyl radical) and digest (release several proteases such as myeloperoxidase, elastase and lysozyme, and glycosidases including N-acetyl-β-glucosaminidase and β-glucuronidase) various pathogens. It is generally believed that leukocytes are indispensable for host defense[8-14].

However, the recruited leukocytes can also func-

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tion as a double-edged "sword". For example, leukocytes recruited to the site of tissue injury, under certain pathological conditions, can cause further tissue damages, leading to deepening, broadening and worsening of tissue injury up to irreversible tissue necrosis and complete loss of functionality. These include, but may not be limited to, ischemia and reperfusion injury (stroke, myocardial ischemia and infarction, and disseminated intravascular coagulation), shock, systemic septicemia, transplantation, severe trauma and burns, acute lung injury (adult respiratory distress syndrome), and various autoimmune disorders (systemic lupus erythematosus, multiple sclerosis and rheumatoid arthritis, etc.)[5].

A multi-step paradigm for leukocyte recruitment

Leukocyte recruitment involves a cascade of cellular events including initial attachment, rolling, weak and firm adhesion, diapedesis, transendothelial migration and chemotaxis. It is now known that at least four families of cell adhesion molecules are involved in the interactions of leukocytes with endothelial cells. They are selectins (CD62) and their glycoprotein ligands, and integrins and their counter-receptors, the immunoglobulin superfamily of cell adhesion molecules[2-19].

The binding of selectins to their cognate glycoprotein ligands is generally believed to be mainly responsible for the initial attachment, rolling and weak adhesion of leukocytes. Consequently, the interaction of integrins with the immunoglobulin superfamily of cell adhesion molecules mediates firm adhesion and signal transduction, which eventually trigger diapedesis and transendothelial migration of leukocytes. The emigrated leukocytes are finally guided by gradients of various soluble chemokines and chemotactic factors to move to their destinations, such as the site of infection or tissue injury.

In regard to the above concept, there have two comments that should be pointed out. First, it is generally believed that the selectins (mainly P-selectin and L-selectin) mediate the first step in the recruitment of leukocytes during inflammation [2-7], [15-19]. Therefore, abrogation of the selectin mediated leukocyte rolling events can prevent further amplification of the inflammatory cascades. Second, little is known so far for the molecular mechanism governing the diapedesis and transendothelial migration of leukocytes[8-14]. Further experimental investigation is apparently required for better understanding of the corresponding molecular networks involved.

A redundant system involved in inflammation

In the past decade, an intensive research activity has been focused on the abrogation of directional leukocyte migration for development of anti-inflammatory medicines. In addition to the extensive in vitro studies, leukocyte adhesion blocking monoclonal antibodies, recombinant proteins, oligosaccharides and their mimetics, many other natural or synthetic inhibitors as well as knock-out and/or transgenic mice for these relevant molecules have been tested in a variety of animal models of inflammation. Almost every currently identified molecule that participates in the directional migration of leukocytes has been in vivo examined with some kinds of promising inhibitory effects on the certain setting or stage of inflammatory responses[2-19].

However, after more than a decade of active investigations, the take-home message appears to be that inflammation involves in a very redundant system; each step or stage of inflammatory responses is usually mediated by coordinating and orchestrating of a repertoire of molecules. Therefore, interruption or abrogation of any given single molecule involved in the directional migration of leukocytes may not be enough for treatment of inflammation. Other molecules can "replace"or "compensate" the roles of the molecule that has been blocked. Anti-inflammatory medicines, especially those of non-steroid/aspirin anti-inflammatory drugs for acute inflammation, continue to be an unmet medical need. In this regard, the recent success of cyclooxygenase II (Cox-II, also referred as prostaglandin endoperoxidase II or prostaglandin G/H synthase II, which is a rate-limiting enzyme for the production of prostaglandin and thromboxanes from free arachidonic acid) inhibitors has alleviated the clinical need, to some extent, for chronic inflammation[20].

Strategies for development of anti-inflammation medicines

For drug research and development, it is a great challenge to deal with the redundant mechanisms or pathways involved in the very complex processes such as those in inflammation. Under these unfa-

vorable "conditions", what are our strategies or plans to conquer this difficulty? How can we cop with it? From my personal point-of-views, the followings may turn out to be the promising directions or "hot spots" for development of anti-inflammatory medicines.

Selectin antagonists for treatment of inflammation

One approach we can take is to develop inhibitors for the critical molecules participating in the early stage of inflammation; that is, to stop the inflammatory responses before they are getting fully amplified and developed[2-7], [15-19]. In this regard, the selectin antagonists are promising candidates, as the current dogma is that selectins are responsible for the earliest events of directional migration of leukocytes (see above). Of course, these also include antagonists for key cytokines, such as tumor necrosis factor-α, interleukins and so on[2-19]. Currently, we are actively pursuing of this line of investigation.

Non-anticoagulant heparin for treatment of inflammation

Alternatively, inhibitors targeting at the multiple molecules at the same time can have a higher probability for effective treatment of inflammatory responses. For example, heparin has various non-anticoagulant activities. Among them are anti-inflammatory roles which include prevention of leukocyte adhesion and activation[21], inhibition of complement activation[22], protection of vascular endothelial cells from a number of damaging substances, such as chemokines, histamine, bradykinin, bacterial endotoxin, lysosomal cationic proteins and oxygen free radicals[23], and maintenance of endothelial wall competence and integrity.

However, the clinical use of heparin for treatment of inflammation is hampered by its inherent strong anticoagulant activity. Therefore, various low molecular weight heparins and chemically and enzymatically modified heparins have been developed. They have relative low anticoagulant activities and remain, to some extent, their anti-inflammatory activities, such as O-desulfated heparin[24] and N-acetylheparin [25].

It is worth noting that heparin has been used as an anticoagulant in clinical practice for several decades. As the result, we know the potential side-effects of heparin and its analogs very well. These include bleeding (usually due to over-dosages), thrombocytopenia (often mild and rarely of any clinical significance), and osteoporosis (develop in a small percentage of patients after receiving 20, 000 units/day of heparin for more than 5 months). Therefore, it is my prediction that the non-anticoagulant heparin may be not only quite safe, but also very efficacious for treatment of acute or subacute inflammation.

NF-κB antagonists for treatment of inflammation

NF-κB is an inducible dimeric transcription factor of the Rel/NF- κB family[26],[27]. This family of transcriptional factors has five cellular members, called NF-κB1 (p105/p50), NF-kB2 (p100/p52), RelA, RelB and c-Rel. All of them have a $\sim$ 300 amino acid domain which shares high homology, termed Rel-homology-domain. The NF-kB complexes are typically localized in the cytoplasm, where they bind to inhibitory cytoplasmic proteins called IκBs. Upon stimulation, IkBs are rapidly phosphorylated and degraded via the ubiquitin-proteasome pathway, resulting in activation and nuclear import of NF-κB.

It is now clearly demonstrated that NF- κ B is a ubiquitous transcriptional factor and a pleiotropic regulator of many genes involved in inflammatory responses. In analogous to the non-anticoagulant heparin, NF-κB antagonists can thus block the up-regulation of many molecules involved in inflammatory responses, such as many cell adhesion molecules, cytokines and interleukines [26],[27]. For example, glucocorticoids can inhibitthe activation of NF-κB by direct interactions with the activated glucocorticoid receptor and NF-κB. Protease inhibitors can inactivate NF-κB by preventing the degradation of inhibitory IκBs. Further, the gene therapy of certain I κB mutants, which are resistant to phosphorylation and degradation, can potently inhibit the activation of NF- κB, thus leading to amelioration of inflammation[28].

CONCLUSION

Directional migration of leukocytes is a cellular phenomenon known for centuries. Systematic and in-depth scientific investigations have dissected and delineated the complex, but fascinating, molecular mechanisms involved in this cellular behavior. This knowledge should facilitate the research and development of effective medicines for treatment of inflammation.

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