Ofer Lider
Gayle G. Vaday
Liora Chalon
Rami heshkoviz
Ehud Baharav
Laura Netzer
Hagai Schor
Amiram Ariel
Suzanne Franitza
Guy Cinamon
Iris Hecht
Idit Altbaum
Alexander Brill

Regulation of immune cell migration through extracellular matrix to sites of inflammation

The Department of Immunology

Tel. +972 8 934 2724 Fax. +972 8 934 2724
E.mail: ofer.lider@weizmann.ac.il

Introduction

Tissue injury caused by infection or physical damage evokes inflammatory reactions and events which are necessary for regaining homeostasis. Central to these events is the translocation of leukocytes, including monocytes, neutrophils, and T lymphocytes, from the vascular system, through endothelium, and into the extracellular matrix (ECM) surrounding the injured tissue. This transition from the vasculature into the site of inflammation elicits remarkable changes in leukocyte behavior, as cells adhere to and migrate across ECM before carrying out their effector functions. Growing evidence suggests that, through its interactions with cytokines and degradative enzymes, the ECM microenvironment place a specialized role in providing intrinsic signals for coordinating leukocyte actions (see Figure 1). Recent advances also reveal that enzymatic modifications to ECM moieties and cytokines induce distinctive cellular responses, and are likely part of the mechanisms regulating the perpetuation or arrest of inflammation. Our laboratory members are studying the dynamic relationships among these factors and how they communicate with immune cells during inflammation.

Intrinsic regulation of immune cell activities in inflamed sites

Since the ECM is a substrate for enzymes secreted by migrating cells, the immunologically relevant context, we suggested, includes not only the intact ECM, but also degraded fragments of ECM components and cytokines. We are currently elucidating the defined molecular structures of breakdown products of ECM and cytokines released from the ECM and the cytokines during cell migration by leukocyte-secreted enzymes. We study whether the enzymatically-formed breakdown products of inflammation might be involved in regulating, in a feedback manner, the inflammatory potential of immune cells. We now have two lines of evidence that support this hypothesis:

1. We found that heparanase, an heparan-sulfate degrading enzyme secreted by migrating leukocytes, can generate immuno-regulatory disaccharide molecules from heparan sulfate and from heparin. These molecules can inhibit TNF secretion from T cells and can modulate chemokine-induced T cell adhesion to and migration through ECM.

2. Elastase, an ECM-degrading enzyme secreted by neutrophils, can not only degrade the pleiotropic cytokine IL-2, thereby modifying its activities, but can also generate small peptides from the cytokine. We have discovered that specific IL-2 peptides regulate cellular functions related to T cell activation (Figure 1).

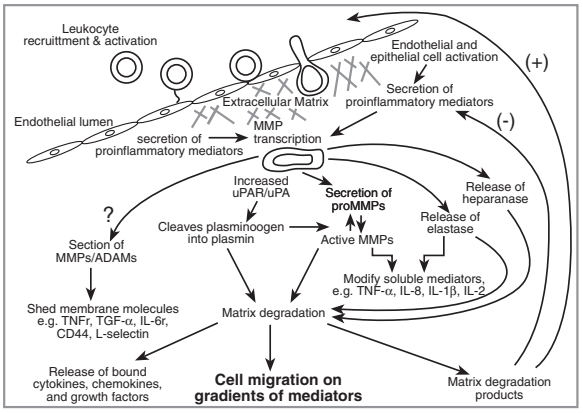

Fig. 1. *Schematic representation of immune cell translocation from the vasculature into tissues.Inflammation induces T cell activation and recruitment to specific loci. Cytokine secretion by T cells, as well as surrounding leukocytes, endothelial, and epithelial cells, evokes the expression and secretion of matrix-degrading enzymes, such as proMMP. uPA-generated plasmin can activate proMMPs. Elastase and heparanase are also secreted, and all of these enzymes degrade ECM to facilitate cell migration. Cytokines and membrane molecules are modified by such enzymes, altering the molecules' function in the context needed. Matrix degradation products participate in a feedback loop to modulate cytokine secretion or further recruit leukocytes. Abbreviations: MMP, matrix metalloproteinase; ECM, extracellular matrix; uPA, urokinase plasminogen activator; uPAR, uPA receptor.*

These findings suggest two important biologic roles:

1. The breakdown products of tissues and cytokines generated by inflammatory enzymes are part of an intrinsic program, and not necessarily molecular waste.

2. The historic encounters of the tissue-invading cells with the constituents of inflammed loci may dictate the cells' behavior upon subsequent exposure to pro-inflammatory mediators. Immune molecules are much more meaningful when they are met within the context of a string of signals. The implication of such a theory is that the inflammatory

milieu should contain not only chemical signals of alert and activation, but also, with time, important negative signalling molecules generated from the context of pro-inflammatory signals.

Regulation of immune cell migration through ECM *in vitro*

Growing evidence suggests that the ECM adjacent to inflammatory loci contains at any given time multiple inflammatory cytokines and chemokines, which act in soluble and ECM-bound forms. Thus, the ECM microenvironment has a specialized role in providing intrinsic signals for co-ordinating leukocyte actions. However, the combined effects of different types of inflammatory signals, chemoattractive and pro-adhesive, that act either alone, sequentially, or simultaneously on migrating T cells has never been studied.

Recently, we designed a novel technique for the real-time analysis of T cells migrating in ECM-like gels along gradients of inflammatory mediators. We are currently using (together with R. Alon; Department of Immunology) this technique to assess the regulation of T cell migration within an ECM environment consisting of chemokine gradients in the context of the cytokine TNF. We now demonstrate that, upon avidly associating with the fibronectin component of the ECM, TNF can markedly inhibit the migration of chemoattracted human T cells towards chemokines, such as SDF-1 or RANTES. This is an example of a non-migratory cytokine capable of regulating the navigation of T lymphocytes in an ECM-associated a chemoattractive environment. We postulate that the primary goal of certain cytokines, such as TNF secreted and integrated into inflamed ECM, may be to localize migrating immune cells to sites of effector functions.

Conclusions and future plans

The activities of immune cells present in extravascular sites, as it becomes increasingly understood, depend on and affected by the context in which these cells migrate and operate. In such a multi-component environment, the migrating immune cell must continuously integrate different signals and adapt and modify its migratory behavior to successfully navigate towards its target site. Hence, in designing the studies done by us, we are studying and will continue to analyze the nature and the mode of action of the migration-altering signals present at sites of inflammation. These signals may originate from (i) the gradual increases in concentrations of the very same migration-inducing chemoattractant, (ii) locally complexed pro-adhesive (and anti-migratory) mediators, and (iii) from the enzymatic break-down products of inflammation. To model and test this complex array of activating signals encountered by tissue-invading T cells, we developed a series of in vitro systems that follows the activation state of cells and their migration. Such means will contribute to our understanding of the actual battle field of the immune system: the tissue itself (i.e. the ECM) and the guiding tools of immunological cells (i.e. inflammatory mediators).

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