Mechanisms of Lymphocyte Accumulation in Pulmonary Disease*

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Lymphocyte accumulation in extravascular, extralymphatic tissue can occur as a result of a limited number of biologic events (Fig 1). Because lymphocytes are capable of cell division, it can be the result of concentration gradient-dependent chemotaxis of distant cells, the result of immobilization of randomly migrating cells, or the result of proliferation of resident and recently attracted cells. In fact, there is evidence that all 3 of these events occur, and because there is usually a phenotypic selected population of cells present in the lungs in disease states, the chemotaxis and immobilization processes are all the more complex. Several sequential events must occur.

The process must begin with initiation of an inflammatory response in the tissue by secretion of growth and chemotactic factors. These and a number of other factors activate and attract distant cells, which must then interact with endothelium via cell and organ specific adhesion molecules, migrate through the endothelium, a process mediated by a separate set of adhesion molecules and active cell motility, and then continue the migratory response along a concentration gradient to the initial site of inflammation. Once there, the cells must have a mechanism of immobilization, either by chemotactic factor induced migratory deactivation, or by nonchemotactic immobilizing factors (which likely act via adhesion molecules). Once attracted to the site of cytokine synthesis, local production of growth factors then permits amplification of the process by proliferation of the selected cell types present.

Of course, the process is much more complex than all of this. There is abundant evidence that there are organ-specific homing mechanisms for T cells which would tend to select out certain subsets of cells capable of migrating into any organ, and more recently, there are data that show that the T cell population in normal subjects capable of both adhering to and migrating through endothelium may be highly selected for recently activated (CD25 +), memory (CD45RO), CD4 + cells. Since there are diseases that are characterized by CD8 + T cell infiltration, there must be mechanisms that alter and activate both the endothelium to be receptive to adherence and transmigration of T cell subsets other than the CD45RO, CD25 +, CD4 + cells noted above, and also, there must be mechanisms that activate CD4 +, CD8 +, natural killer (NK), and other T cells to assume a migratory posture. Last, there are clearly mechanisms that selectively inhibit the migration of some, but not other T cell subsets.*

Lymphocyte Chemotactic and Growth Factors

This review will concentrate on only 1 of these many aspects of selective lymphocyte accumulation, the role of chemotactic factors for T cells. Four concepts will be discussed. The first is that growth factors for T cells are invariably T cell chemotactic factors. Second, the 2 functions are mediated via the same cell surface high affinity receptors, thus providing a convenient duplication of function for a single molecule and receptor; both functions serving the greater goal of amplification of T cell accumulation. Third,

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that the T cell has further conservation of structural features by utilizing the same cytoskeletal apparatus for migration in G$_d$ and G$_c$ as it does for the mitotic spindle in G$_d$ + M; thus separating the functions by cell cycle phase. Fourth, we will describe how by using these principles, we were able to clone a very novel T cell chemotactic factor that utilizes CD4 as its receptor. In the course of this discussion we will define this interleukin as a pure competence-type growth factor; and thus suggest new functions for CD4. Through the use of natural ligands like this one, we have begun to dissect the early phases of the CD4+ T cell cycle by defining a true competence/progression break point in G$_d$.

In order to accomplish these tasks, we will first define the paradigm of growth factor-chemotactic factor equality by reviewing some of the data that demonstrate that both traditional and nontraditional T cell growth factors all have inherent chemotactic activity. The chemotactic activities of antigen, IL1, IL2, insulin, and insulin-like growth factor 1 (IGF1) will be used as models.

When the T cell growth factor (IL2) was first described, our laboratory had been studying T cell migration for several years, but at the time still lacked well defined naturally occurring factors that interacted with the T cell via defined receptors. For all these reasons, we undertook a series of experiments to determine if IL2 was chemotactic for T cells.$^{16}$ Resting cells (G$_d$) had no chemotactic response to IL2, in concert with the findings that all the biologic effects of IL2 are mediated via high affinity heterodimeric receptors. Once induced into G$_c$, T cells migrated to IL2, a response that was inhibited by anti-Tac antibodies and could be prevented by the inhibition of p55 IL2r synthesis and expression. The response was concentration gradient dependent. Of particular interest at the time was that the lymphocyte chemotactic factor (LCF), a less well defined molecule at that time,$^{22}$ induced complete responses in G$_c$ cells, a response that was not inhibited by cell activation to G$_d$.

These findings stimulated Miossec et al.$^{23}$ and others$^{24}$ to demonstrate that IL1 was chemotactic for T cells, and we and others have demonstrated that it stimulates the response of both CD8 and CD4 cells. Wilkinson et al.$^{25}$ had previously suggested a role for antigen itself in T cell migration, however, his experiments were never completely free of the possibility that antigen-induced cytokines may have played a role. This led his group$^{26}$ and others, including our laboratory, to demonstrate that antibodies to CD3 were potent chemotactic factors for T cells. Of particular interest in these experiments was the absolute requirement for the presence of accessory cells, which can function either live or formalin-fixed to augment the T cell migratory response. While membrane-bound IL1 may explain part of the CD3 mediated T cell migratory response, a component of the anti-CD3-induced motility is not reproduced by IL1 nor inhibited by anti-IL1 antibodies that some other cell-cell interaction plays a role also.

While all of these data suggested that there might be an intimate relationship between growth factors and chemotactic factors, our studies using traditional hormonal growth factors were most helpful in confirming this hypothesis. We first investigated the chemotactic response of T cells for insulin.$^{27}$ Insulin receptors on T cells have been well characterized, and in fact are a part of the family of growth factor receptors and cell surface molecules that are induced in early G$_c$ of the T cell cycle. Using this information, we demonstrated that insulin is chemotactic for activated T cells at concentrations usually attributed to hormonal actions. The response was concentration gradient-dependent. During those experiments, we demonstrated that high concentrations of insulin could induce motile responses in resting T cells. Since insulin can interact with the IGF1 receptor at high concentrations, we used this chemotactic finding to hypothesize that resting T cells had functional IGF1 receptors.$^{27}$ In fact, we found that the IGF1 receptor in a form identical to its structure on other cells is expressed on resting T cells, and we could attribute all of the high dose insulin effect in resting cells to IGF1 receptors.$^{27}$

The paradigm would not be complete, however, unless both insulin and IGF1 were true growth factors for T cells. In keeping with its receptor induction on resting cells, insulin is an IL2-like progression factor for T cells (although obviously not selective in this capacity, nor particularly potent). The IGF1 receptors are present on resting G$_d$ T cells, and IGF1 binds with similar kinetics to both resting and activated cells. It is mainly a growth factor for T cells which is almost as potent as IL2.$^{27}$ Of course, since these early studies, many other interleukins have been demonstrated to have chemotactic activity for T cells, although thorough evaluation of the growth and chemotactic activity via the same receptor has not yet been published.

Throughout all these studies, our laboratory had been studying the biologic functions of another T cell chemotactic factor, lymphocyte chemoattractant factor.$^{28}$ We had originally defined this cytokine for its chemoattractant activity for T cells in order to obtain a well characterized natural chemotactic factor. Its chemical characteristics distinguished it from other known cytokines and interleukins, and subsequently molecular cloning and expression of recombinant protein has corroborated early impressions that it is a unique gene product and protein. After identification as a T cell chemotactic factor, it was next discovered that its activity was selective for CD4+ cells.$^{29}$ The LCF induced migratory responses in T cells in G$_d$ and G$_c$ equally,$^{30}$ suggesting that its receptor was present on both resting and activated CD4 but not CD8 cells. Studies that showed that all its activities were inhibited by Fab of monoclonal anti-T4 antibodies$^{31}$ suggested that its receptor could be CD4 itself. Induction of migratory responsiveness in L3T4(-) mouse hybridoma cells by transfection with normal human CD4 strengthened this concept. All LCF induced activities in these hybridomas were dependent upon the presence of the intracytoplasmic signalling component of CD4. Studies demonstrating that monocytes$^{28}$ and eosinophils$^{31}$ utilize their surface CD4 as chemotactic factor receptors for LCF suggest that CD4 can act independently of the TcR and CD3.

We subsequently demonstrated that LCF interaction with CD4 induced a rise in intracellular Ca$^{2+}$ and phosphotidyl inositol turnover into IP$_3$, further suggesting a ligand-receptor-like relationship. These signalling events were reproduced in the CD4 transfected mouse hybridoma cells noted above; once again, inhabitable by Fab of anti-T4 monoclonal antibodies, and by recombinant soluble CD4.

In keeping with the hypothesis that growth factors are chemotactic factors also, we looked for growth factor activity...
for LCF. It induces IL2r, insulin receptors and MHC class II (HLA-DR) molecules on CD4+ T cells, and markedly enhances RNA synthesis. It does not stimulate IL2 secretion or synthesis, and in concert with this finding, it does not induce DNA synthesis whether measured by thymidine incorporation, acidic orange, or propidium iodide staining. Thus, CD4 can act independently of the TcR/CD3 complex as a competence type growth factor. Since the LCF-CD4 interaction induced G1→G2 transition, we looked for a costimulatory effect with IL2 and insulin, both of whose receptors are induced in G1. LCF via CD4 establishes insulin and IL2 induced migratory responsiveness in association with receptor induction, and makes the T cell competent for an IL2 growth signal to continue through the cell cycle to DNA synthesis and division (unpublished data, W.W. Cruikshank, Ph.D., 1992).

Summary and Speculations

However, the question that arose from all these studies was whether the migratory response is ever separable from cell cycle events. Is migration an epiphenomenon of cell activation? We think not because of all the data on chemotaxis of nondividing cells and because antibodies to CD4 induce motile responses but have no effect on IL2r, MHC II expression, insulin, or transferrin receptors, nor on intracytoplasmic signalling events. Our laboratory has demonstrated, however, that the HIV-1 envelope protein gp120 can act as a CD4 ligand to induce signalling, migration, and cell cycle. Thus CD4 provides a unique receptor model for which we have growth factor natural ligands and artificial antibodies that induce only the migratory response without the cell cycle changes.

It is well known that the T cell migratory response utilizes a number of cytoskeletal proteins, many of which are also part of the mitotic spindle. Thus, it appears that the cell is able to use a single ligand-receptor interaction to induce early cell cycle events and motility, while late cell cycle events and cell division must await availability of structural apparatus before proceeding. Precisely which signalling events are common to the entire cell cycle progression and which are unique to early and late events have yet to be determined. However, natural ligands like LCF which induce competence only, and artificial ligands for the same CD4 receptor like anti-CD4 antibodies should help to determine the phosphorylation events and nuclear signalling pathways necessary and essential for the T cell growth cycle.

Regarding the special case of the lung, the localization of selected T cell subtypes at inflammatory sites is well documented in antigen-induced models. In sarcoidosis, where the antigen is unknown, one of the earliest local lung observations was the presence of CD4+, IL2r+ which spontaneously proliferated and secreted IL2. And most recently, there is significant indirect and direct evidence that chemotactic factors may play an important role in the inflammatory process in the lung in hypersensitivity pneumonitis.

The simple hypothesis of chemoattraction to sites of chemotactic factor synthesis and secretion invoked for inflammatory responses associated with nondividing leukocytes must be reevaluated for the much more complex circumstance of accumulation and localization of dividing leukocytes like T cells in a very complex immunologic organ like the lung. We have just begun to understand the coordinated mechanisms of chemoattraction and proliferation at the cellular level. Hopefully this information will help us design approaches to favorably alter T cell-specific accumulation in extralymphatic tissues through the use of reagents that effect all the stages of accumulation: chemotactic factor synthesis and secretion, target cell binding of chemotactic factors, adherence and transmigration of T cells through endothelium, migration of cells through organ matrix, and finally local cell proliferation.

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In Situ Regulation of Pulmonary Macrophage TNF-α mRNA Expression by IL2*

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The antitumor activity of IL2, used either alone or in combination with the adoptive transfer of activated lymphocytes, is well known.1,3 However, the use of IL2, a 15 kd glycoprotein produced by activated T lymphocytes, as an antitumor agent has been limited by severe dose-dependent toxicities, including a diffuse vascular leak syndrome and shock.* Indeed, the pathophysologic consequences of high-dose IL2 are remarkably similar to those seen in septic shock. Several recent investigations have identified tumor necrosis factor-alpha (TNF-α) as an important mediator of toxicity in endotoxemia and Gram-negative sepsis.** The clinical similarities between IL2 toxicity and sepsis suggest that TNF-α may be a mediator of some of the toxic effects associated with IL2.

Several lines of evidence support this hypothesis. For example, IL2 induces TNF-α expression in lymphocytes and macrophages, and studies have documented circulating TNF-α in patients receiving IL2. Also, mice receiving high-dose IL2 have significantly longer survival if treated with anti-TNF-α monoclonal antibody. While it has been suggested that the pulmonary vascular leak syndrome accompanying IL2 administration may be due to the in situ generation of TNF-α, the in situ upregulation of TNF-α expression within the lung has not previously been demonstrated. We hypothesize that upregulation of TNF-α in pulmonary macrophages in response to IL2 contributes not

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