[Frontiers in Bioscience 9, 3156-3162, September 1, 2004]

MECHANISMS OF INFLAMMATION: THE GOOD, THE BAD AND THE UGLY

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1. ABSTRACT

To the general public, the term "inflammation" is associated with pain, swelling, fever and a general sense of unease ranging from mere nuisance to debilitating illness. Under normal circumstances, the process of inflammation is actually a protective response designed to ward off invasion of the person by pathogens such as bacteria, viruses and/or parasites. The immune system of higher mammals (e.g. humans) is comprised of two distinct "arms" termed the innate and the adaptive systems. While these two components play unique roles in controlling pathogens, each relies, in some part, upon the effective function of the other in order to efficiently eliminate invading microorganisms. There are however situations in which this complex system is unable to properly function leading to unresolved infections and/or chronic states of inflammation. This review will summarize the basic mechanisms involved in the inflammatory process as well as discuss some of the key mediators and modulators of this process.

2. INTRODUCTION

The human body is under constant assault by pathogens in the environment that could potentially cause harm and even death if given access to the blood, organs or interstitial spaces. Fortunately, higher organisms have developed efficient means of restricting exposure to microorganisms; chiefly the epidermal and mucosal barriers. However, in some instances these barriers are not sufficient to prevent potential pathogens from gaining invading the body (e.g. breaks in the skin or perforation of the intestinal wall). Once the organism gains entrance to the blood and/or interstitium, it must be recognized as "foreign", contained and then eliminated from the body in order to prevent illness and possibly death. Specialized cells in the bloodstream (e.g. leukocytes) have developed to carry out these necessary responses to invading organisms. There is a "division of labor" within leukocytes when dealing with potential pathogens. That is, some leukocytes are responsible for the recognition of a "foreign" antigen (e.g. antigen presenting cells such as dendritic cells), others serve to engulf invading bacteria (e.g. phagocytes) and still others serve to produce soluble mediators that assist in recruitment of other leukocytes to the point of entry or assist in the elimination of antigens (e.g. T-cell derived cytokines and B-cell derived immunoglobulins). Together these specialized leukocyte populations assist one another in the elimination of invading pathogens from the body.

All microorganisms that can potentially cause disease enter the body via the respiratory or alimentary tracts or through wounds in the skin. The pathogen and/or its secreted antigens are taken up by specialized cells known as dendritic cells that are recruited to the site of infection in the tissue. Dendritic cells function specifically to ingest antigens and "transport" them to the draining lymph node, Macrophages can also perform this role but they are usually less efficient than dendritic cells. In cases where the surface Ig on a B cell can bind the antigen, then B cells can also function as an "antigen presenting cell" (APC). These three cell types, dendritic cells, macrophages and B cells are often referred to as "professional" APCs. These "professional APCs" are the only cell types to express MHC class II molecules. The uptake of antigen in the tissues is aided by the outcomes of the innate immune response (e.g. inflammation). During the first encounter with such antigens (i.e. a primary immune response) T cells capable of recognizing these antigens are stimulated to proliferate and generate effector T cells such as $CD8^+$ CTL, $CD4^+$ Th1 or Th2 cells and memory cells (Figure 1). This step is known as "priming". There are some differences between the activation of naïve T cells (i.e. those that have never seen antigen) and effector T cells. The major difference is that naïve T cells are very dependent on a co-stimulatory signal that is independent of TCR mediated signaling. Signaling through the TCR complex is often referred to as "signal 1" whereas the costimulatory signal is known as "signal 2". The co-stimulatory signal is delivered to the T cell by engagement of the T cell molecule CD28. The ligand for CD28, the"B-7" molecule is expressed only on the professional APCs (Figure 2). The expression of B-7 on these cells can be upregulated by the uptake of antigen. Thus, only professional APCs can activate naïve T cells because they express B-7 and thus can deliver signal 2 to the T cell. Dendritic cells (so named because of their appearance) that are present in the tissues, such as beneath epithelial surfaces or in solid organs, are at an "immature" stage. Immature dendritic cells are very efficient at the uptake of microbial pathogens because they possess receptors (e.g.. DEC-205 and the Toll Receptors) for molecules unique to microbial surfaces. They are also able to engulf soluble antigens by micropinocytosis. Immature dendritic cells are not potent antigen presenting cells for naïve T cells because they do not express the co-stimulatory molecule B-7. Upon antigen uptake and transport to the lymph node, the dendritic cells mature. They now express B-7 molecules but are no longer able to

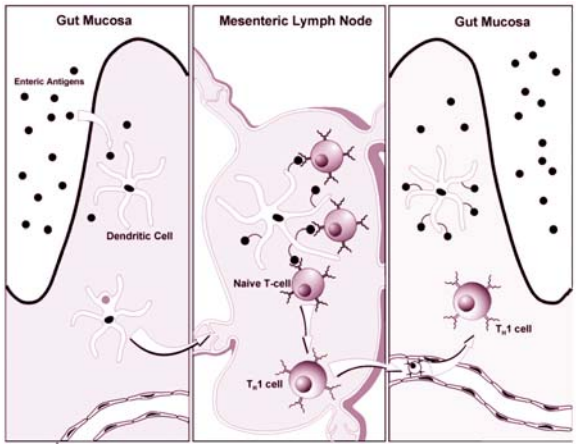

Figure 1. Dendritic cells or other APCs engulf and process antigen from the site of entry (in this case the gut mucosa) and subsequently migrate to a local lymph node where they then influence the polarization and proliferation of naïve T-cells into reactive effector cells. These effector cells then reenter the circulation and home to the site of antigen entry where they again encounter their APC-associated antigen. This second encounter with antigen then activates the T-cell to produce soluble mediators of inflammation such as cytokines and chemokines.

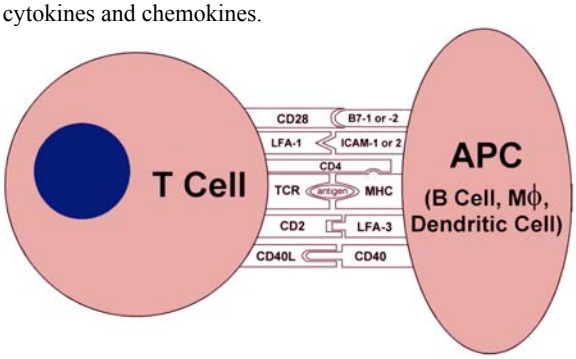

Figure 2. T-cell/APC interactions require not only engagement of the T-cell receptor by MHC-bound antigen but also co-stimulatory signals such as CD28 and CD40/CD40L for full activation of both APC and T-cell.

take up more antigens. In addition to bacteria, dendritic cells are also likely to be the primary APC for initiating immune responses to viruses and fungal pathogens.

It is well appreciated that macrophages can be activated to have potent anti-microbial activity. In addition they are also potent APCs following phagocytosis. Like immature dendritic cells, macrophages have receptors that can bind to surface molecules on the surface of bacteria. These receptors include the mannose receptor, the "scavenger" receptor, complement receptors and Toll-like receptors (TLR). Uptake of bacteria through these receptors can elicit the production of cytokines by these macrophages. Similar to immature dendritic cells, resting macrophages do not express the B-7 molecules and thus cannot activate naïve T cells. The uptake of microbial pathogens induces the expression of the B-7 co-stimulatory molecules. In the absence of microbial uptake, macrophages and dendritic cells express peptides from proteins present in the tissue. Some of these tissue derived peptides will also be present in the thymus and T cells that would be capable of recognizing such antigens are deleted (by negative selection) in the thymus. However some of these peptides are not expressed in the thymus and therefore T cells that could recognize these tissue specific peptides are likely to exist in the periphery. Naïve T cells that were capable of recognizing these tissue specific peptides could be stimulated by dendritic cells and macrophages if such cells expressed B-7. The absence of B-7 molecules on these APCs in the absence of pathogens is thus important for the prevention of autoimmunity. B cells are also included as "professional" APCs because they can take up antigen if it binds to Ig on the surface of the B cell. Because B cells take up antigen through their antigen receptor, which is expressed at high levels, they have the potential to express a high density of the antigen on the cell surface. As with the professional APCs, B cells do not constitutively express B-7, however B cells that have taken up microbial antigens do express B-7. It is not clear how important B cells, compared to dendritic cells and macrophages, are for activating naïve T cells. B cells that express germline encoded Ig genes prior to affinity maturation via somatic point mutation may not have very high affinity and thus may not be as potent as dendritic cells and macrophages in the uptake of microbial pathogens.

Professional APCs are distributed in different areas within the lymphoid tissues: dendritic cells are in the cortical areas and in particular are widely dispersed in the areas enriched in T cells; macrophages are concentrated around the sites of supply and drainage of cells to and from the lymph node; B cells are found mainly in the follicles. T cells enter the lymph node through the high endothelial venules. The T cells that contact an APC expressing an antigen recognized by its TCR remain in the lymph node where they proliferate and generate effector T cells (Figure 1). After a few days some of the effector cells are returned to the circulation, via the lymphatics, so that they may traffic through the body to seek out other areas of infection with that pathogen. T cells that do not recognize any of the presented antigens are rapidly returned to the circulation via the lymphatics.

Stimulation of naïve T-cells by professional APCs drives the T-cell into the cell cycle. In addition it stimulates the production of IL-2 and the expression of the α chain of the IL-2 receptor (also known as CD25). The IL-2 receptor is a heterotrimeric complex of an α, β and γ chains. A "lower affinity" dimeric form of the receptor consisting of the β and γ chains is expressed on resting T-cells such that resting T-cells can bind IL-2 in the presence of high concentrations of the cytokine. The production of the IL-2 receptor α chain leads to the expression of a high affinity receptor that can respond to lower amounts of the cytokine. This enables a T-cell to synthesize enough IL-2 to sustain its own proliferation (autocrine growth). After several days of sustained proliferation a single T-cell can be amplified to produce several thousand progeny cells. In addition to replicating, this growth phase is also accompanied by differentiation events that enable the T-

cell to acquire the effector functions associated with activated T-cells. The pathways provided by signal 2 augment the production of IL-2. This is accomplished by an increase in the stability of the mRNA encoding IL-2. In addition signal 2 activates the transcription factors, c-Jun which together with fos (activated by signal 1) forms a transcription factor complex known as AP-1. The AP-1 complex, in combination with the NFAT (nuclear factor of activated T-cells) increase IL-2 transcription. Together these 2 effects (mRNA stabilization and activation of NFAT and NF-κB) increase IL-2 production up to 100-fold.

The trafficking of T-cells to particular sites in the body is influenced by several distinct families of molecules including selectins and integrins. Each member of the family has a particular ligand either on endothelial cells or on APCs. The selective expression of the ligand for these molecules by particular tissues can influence the trafficking of lymphocytes. Lymphocyte trafficking and homing is also influenced by the secretion of cytokines and chemokines at sites of infection. As previously noted, T-cell signaling occurs through the TCR/CD3 complex. This is often referred to as signal 1. Naïve T-cells require more than signal 1 for activation, they need to be stimulated through a molecule known as CD28. This is commonly referred to as signal 2. The ligand for CD28 is the B-7 molecules. There are actually 2 B-7 molecules B-7.1 (CD80) and B-7.2 (CD86). The B-7 molecules are homodimers that are expressed exclusively on "professional APCs". Interestingly the expression of B-7 molecules on immature dendritic cells and macrophages is very low. The expression of B-7 molecules is upregulated during maturation of these cells and upon interaction with T cells. During T cell interaction with APCs, the receptor CD40 on the APC is engaged buy a T cell molecule known appropriately as CD40Ligand. On mature T cells CD28 engagement (i.e. signal 2) also provides stimulatory activity. CD28 engagement is essential to augment IL-2 production by Th1 cells (Figure 2). A structurally related molecule to CD28 that also binds to the B-7 molecules is CTLA-4. CTLA-4 is not expressed on resting cells but gradually appears on activated cells. CTLA- 4 has greater affinity for the B-7 molecules than CD28. Therefore it is believed that when CTLA-4 is expressed (e.g. on activated T cells), the B-7 molecules bind exclusively to CTLA-4 and not to CD28. Unlike CD28, CTLA-4 is not coupled to the production of IL-2. Therefore the binding of CTLA-4 to B-7 molecules shutdowns the production of IL-2 which is a growth factor essential for T cell expansion. In addition to ablating signal 2 delivery through CD28, the expression of CTLA-4 may also deliver "negative" signals that further prevent the expansion of these antigen specific T cells. Thus expression of CTLA-4 is often thought of as a means to "switch off" the immune response to a specific antigen once it has been eliminated.

Based upon their patterns of cytokine production, murine and human T-helper cells are divided into several additional subsets termed Th1, Th2, Th0, Th3, Thp and Tr1 cells (25). Activated Th1 cells secrete interleukin-2 (IL-2), interferon gamma (IFN-γ) and lymphotoxin-α (LT-α), whereas Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. Th0 cells synthesize and release both Th1 and Th2 cytokines while Th3 cells produce large amounts of transforming growth factor-β (TGF-β). Evidence is accumulating that both Th1 and Th2 cells are derived from a common IL-2 secreting precursor T-cell termed Thp cells. There is now good evidence demonstrating that macrophage or antigen presenting cell (APC)-derived IL-12 is crucial for inducing Th1 differentiation whereas IL-4 is required to promote the differentiation of Thp cells into Th2 cells (25). A number of different studies have shown that Th1 cells are involved in an immune-mediated inflammatory response termed delayed type hypersensitivity (DTH; Type IV Hypersensitivity). This type of cell-mediated immunity (CMI) is the primary defense against certain infectious agents such as intracellular bacteria, fungi and protozoa. This protective immune response involves not only activation of Th1 cells and the subsequent release of their cytokines but also involves Th1 cytokine-mediated activation of macrophages and other phagocytic leukocytes that release additional pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8, IL-12 and IL-18. On the other hand, a Th2-type response is required for humoral immune responses (i.e. antibody production) as well as effective protection against extracellular pathogens such as helminths.

The first step in mounting an effective immune response against antigens within the intestinal and/or colonic interstitium is the selective migration (i.e. homing) of naïve T-cells from the peripheral circulation into the Peyer's Patches or mesenteric lymph nodes draining the gut. This selective and highly efficient extravasation process is accomplished by the specific interaction between T-cell-associated L-selectin and its ligand (GlyCAM-1) located on specialized endothelial cells in lymphoid tissue called high endothelial venules (HEVs). In addition, naïve T-cells may also home specifically to the gut mucosa via the interaction of T-cell associated α4β7 integrin and HEV expressed mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (4;5;9;18;19;28;33). If the naïve T-cell encounters its specific antigen (Ag) presented on the surface of APCs in association with major histocompatability complex class II (MHC II), the T-cell will bind the MHC II-Ag complex via its T-cell receptor (TCR) complex and become activated. During this local activation process the T-cell will proliferate, shed its L-selectin and enhance surface expression of certain adhesion molecules such as LFA-1 (CD11a/CD18), VLA-4 (α4β7), VLA-5, VLA-6 and CD44 (1;7;16;23;26). The progeny of these activated T-cells may differentiate into effector (e.g. Th cells) or memory cells. Unlike effector cells, memory cells may exist for long periods of time (20 years) in the absence of antigen (Ag) stimulation (1). These lymphocytes do not produce effector molecules, e.g. cytokines, unless they are stimulated by antigen. Following the initial activation of T-cells within the lymphoid tissue, effector or memory cells re-enter the circulation via the efferent lymphatics and the thoracic duct.

Upon return to the systemic circulation, activated T-cells will no longer efficiently home to lymphoid tissue

due to loss of surface L-selectin. Instead, these cells will preferentially now home to sites of infection/inflammation which are usually sites where the offending Ag originally gained access to the tissue (i.e. the gut). This new pattern of homing is mediated by the interaction between lymphocyte-associated VLA-4 ($\alpha_4\beta_1$), LFA-1 and CD44 and venular ECAMs (VCAM-1, ICAM-1 and –2), and hyaluronate, respectively (1). Thus, effector and memory T-cells possess a different homing pattern than do naïve cells. Once extravasated, an effector or memory T-cell may re-encounter its specific Ag via the specific binding of its TCR to Ag bound by the MHC II complex expressed on APCs such as dendritic cells, macrophages and possibly endothelial cells. This second antigen-specific interaction activates lymphocytes to synthesize and release IFN-γ and IL-2. IL-2 promotes clonal expansion of T-cells and enhances the function of helper T-cells and B cells, whereas IFN-γ interacts with and activates APCs and macrophages to produce IL-12 (32). IL-12 then feeds back onto the T-cells to further enhance production of IFN-γ and promote the differentiation of these T-cells to Th1 cells capable of producing even larger amounts of IFN-γ and IL-2. IFN-γ can then activate endothelial cells and enhance ECAM expression on the post-capillary venular endothelium. In addition, IFN-γ-activated macrophages produce large amounts of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, IL-12 and IL-18 as well as reactive oxygen and nitrogen metabolites (e.g. superoxide, hydrogen peroxide, nitric oxide). All of the above mentioned mediators are thought to be important in recruiting and activating phagocytic leukocytes such as PMNs and monocytes/macrophages for the efficient destruction of the invading pathogens.

The potential for the immune system to produce local and even systemic injury suggests that healthy individuals possess mechanisms that limit the immune response and in some cases completely suppress it. It has been suggested that specific subsets of $CD4^+$ T-cells collectively referred to as Treg cells accomplish this task (22;27). This immuno-regulatory group of T-cells is composed of: a) a naturally-occurring $CD4^+CD25^+$ subset called Treg, b) T regulatory-1 cells (Tr-1) produced by repetitive antigenic stimulation of isolated $CD4^+$ T-cells (human or mouse) in the presence of IL-10 *in vitro* and c) Th3 cells produced by oral exposure to antigen (22;27). All three of these $CD4^+$ regulatory subsets contain cells capable of inhibiting a variety of autoimmune disease models including chronic colitis (11;12;22;27). It is becoming increasingly appreciated that IL-10 and TGF-β are two key components required for the regulatory function of Treg cells *in vivo*.

A recent series of studies by Groux and coworkers have characterized specific clones of $CD4^+$ T-cells that differ substantially from the classical Th1 and Th2 cells. These cells are generated by repetitive antigenic stimulation of mouse or human $CD4^+$ T-cells in the presence of IL-10 *in vitro* and are termed T regulatory-1 (Tr-1) cells (11). These regulatory T-cells have been shown to be immunosuppressive in different models of immune-mediated inflammation. Indeed, Tr-1 cells are capable of attenuating established colitis in SCID mice reconstituted with $CD4^+CD45RB^{high}$ T-cells (11). Whether or not Tr-1 cells are actually generated *in vivo* and whether they act to inhibit the activation, polarization, recruitment or effector functions of pathogenic cells contained within the $CD4^+CD45RB^{high}$ population is not known at the present time. It is thought these regulatory cells exert their protective effect *in vivo* by the elaboration of large amounts of IL-10. This regulatory cytokine functions to inhibit the synthesis of Th1 and/or macrophage-derived cytokines by different populations of leukocytes (13;22). In addition, IL-10 promotes the differentiation of TGF-β producing Th3 cells *in vitro* and attenuates the formation of macrophage-derived reactive oxygen and nitrogen metabolites (13;25). Finally, IL-10 has been shown to inhibit surface expression of MHC Class II molecules and intercellular adhesion molecule-1 (ICAM-1).

In addition to the *in vitro* generation of Tr-1 clones, investigators have identified a naturally occurring subset of Treg cells enriched within the 10% of peripheral $CD4^+$ T-cells that express CD25 (21;24;29;31). This population of Treg cells was originally identified by its ability to attenuate the development of autoimmune gastritis following neonatal thymectomy or by the transfer of specific subsets of $CD4^+$ T-cells into athymic nude mice (24;31). Since then, $CD4^+CD25^+$ T-cells have been shown to inhibit other autoimmune disorders including diabetes and colitis (24;31). The mechanisms by which these Treg cells suppress autoimmune tissue injury remain the subject of active investigation. Although initial *in vitro* studies suggested that the suppressive activity of $CD4^+CD25^+$ T-cells was not dependent upon IL-10 or TGF-β (22;27), subsequent *in vivo* investigations have demonstrated that IL-10 and TGF-β are required for $CD4^+CD25^+$ T-cell mediated suppression of colitis induced in SCID mice reconstituted with $CD4^+CD45RB^{high}$ T-cells suggesting that IL-10 is in fact required for the suppressive activity of these cells *in vivo* (3). In addition, Read *et al* have shown that the suppressive effects of $CD4^+CD25^+$ Treg cells in attenuating colitis *in vivo* is also dependent upon both CTLA-4 and the production of TGF-β (21). Taken together, these data suggest that both IL-10 and TGF-β play important roles in Treg suppression of colitis *in vivo*.

A third subset of $CD4^+$ T-cells that is thought to regulate Th1-type immune responses is the Th3 T-cell population. These cells are generated by oral administration of antigen and have been shown to produce large amounts of TGF-β and varying amounts of other Th2–type cytokines such as IL-4 and IL-10 (8;25;30). TGF-β is a pleiotropic cytokine that has been shown to be important in down-regulating the excessive inflammation observed in a variety of experimental mouse and human immune based diseases including IBD (17;20;25;30). Although the biological activity of TGF-β is complex, in general it appears to inhibit the generation of Th1 cells, IL-12 production and cell-mediated immunity *in vivo* (25;30). Previous studies have suggested that Treg cells possess T cell receptor (TCRs) specificity identical to that of the effector T cells they regulate, more recent work suggests that prior exposure to bacterial antigens is not required for

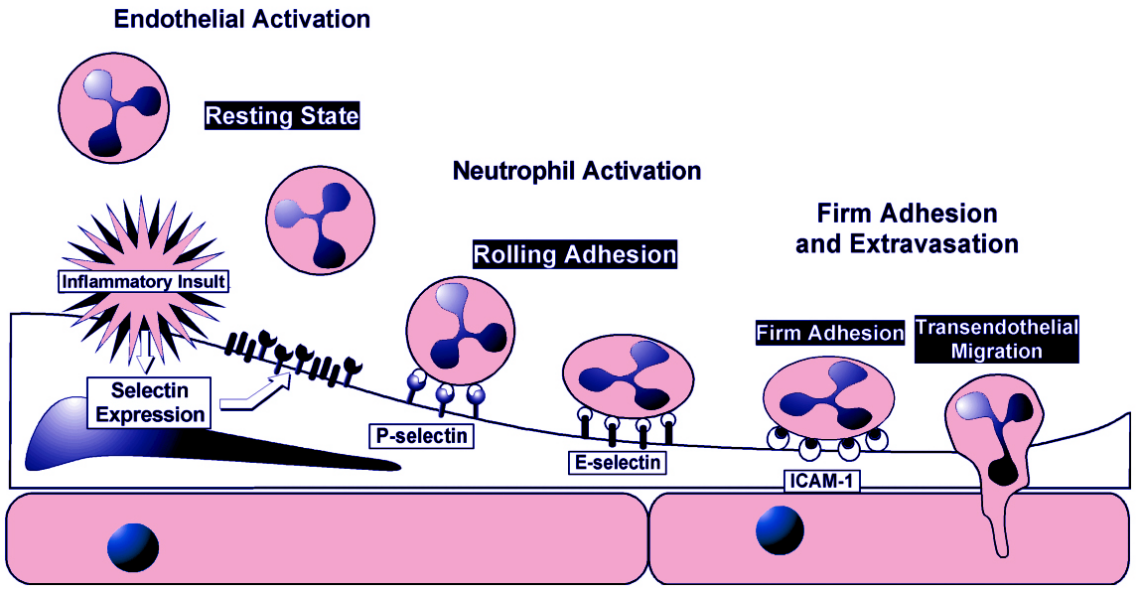

Figure 3. The "3 step" paradigm of leukocyte recruitment from the circulation. Leukocytes first tether to endothelial-expressed selectins and begin rolling. Following rolling-induced activation, leukocytes then become firmly adhered via interactions with ICAM-1 and VCAM-1 and finally migrate into the surrounding interstitium.

Treg cells to exert their protective effect in the $CD4^{+}CD45RB^{high}$/SCID mouse model of colitis (2). These observations suggest that the peripheral T-cell pool contains regulatory T-cells that control or limit intestinal inflammation and injury induced by immune responses to luminal antigens. Not surprisingly, animals rendered deficient in either IL-10 or TGF-β lack the appropriate regulatory cell responses and will spontaneously develop systemic and/or localized inflammation including colitis.

Now we turn our attention to the process of leukocyte recruitment from the circulatory system to the interstitium and/or site of the inflammatory stimulus. The process of leukocyte recruitment into surrounding tissues follows a general "3 step" paradigm that applies not only to extravasation of leukocytes under inflammatory conditions but is also believed to apply to homeostatic leukocyte trafficking (Figure 3). The steps of the leukocyte recruitment paradigm all involve coordinate interactions between the leukocyte to be recruited and the vascular endothelium. For example, the first step in leukocyte recruitment is the tethering of circulating leukocytes to the adjacent endothelial cells that induces rolling of the leukocyte along the vascular surface. This rolling slows the leukocyte and is mediated through the interaction of endothelial selectins and leukocyte-derived ligands (e.g. endothelial P-selectin and leukocyte PSGL-1). Other selectins involved in the rolling process include endothelial-derived E-selectin as well as leukocyte-derived L-selectin. In addition to slowing the velocity of the leukocyte, rolling also induces an activation event in the leukocyte that induces the next step in the recruitment paradigm; that is firm adherence of the leukocyte to the vascular endothelium. It is thought that the activation event mentioned above occurs when the rolling leukocyte encounters a chemoattractant molecule that is either present as a soluble bacterial-derived gradient or presented on the vascular endothelial surface. Some of the molecules capable of acting as chemoattractant activators for leukocytes include bacterial products (e.g. fMLP), activated complement proteins, lipid-derived mediators (e.g. platelet activating factor; PAF), chemokines and other molecules (6;10;14;15). Indeed, chemokines have recently become the subject of intense study as mediators of leukocyte recruitment. Specifically, chemokines such as monocyte-chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), thymus and activation-regulated chemokine (TARC) and RANTES are thought to be critical for adhesion of monocytes (6). Recent work has also indicated that specific chemokine receptors are responsible for imparting specificity of the different steps in the recruitment of leukocytes. The subsequent firm adherence of leukocytes to the endothelium is mediated via the interactions between leukocyte-derived β_1 and β_2 integrins and members of the immunoglubulin superfamily expressed on the endothelial surface (e.g. ICAM-1 and VCAM-1). Finally, there is the poorly understood extravasation and movement of leukocytes into the interstitial spaces that is thought to be mediated by other members of the integrin family.

In summary, inflammation is clearly a complex process involving many different aspects of both the immune and circulatory systems and serves to protect the host from invasion by potential pathogens.

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Key Words: Adhesion Molecules, Leukocytes, Inflammation, Cytokines, Lymphocytes, Review

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