Lymphocyte Adhesion and Autoimmunity

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Abstract

Aberrant lymphocytes signaling is one of the numerous mechanisms thought to be responsible for the pathogenesis of autoimmune diseases. One of the most successful approaches to the treatment of autoimmunity is through targeting of lymphocytes, whose multiple targetable functions include proliferation, cytokine secretion, and migration. The adhesion process is a critical step, not only for migration but also for their communication with antigen-presenting cells, and is therefore a clear target for therapy. This manuscript will discuss the migration of T cells, which are at the heart of many autoimmune responses. We will review the importance of increasing our comprehension of these events, focusing on migration since they enclose a multitude of potential therapeutic targets for autoimmunity. The interface between lymphocytes and antigen-presenting cells and the formation of the immunological synapse will be reported in detail. We will address the following questions: What enables T cells to migrate to sites of injury, and what are the options to intervene? What is the contribution of co-receptors to T cell adhesion? How can we manipulate this knowledge for therapeutic purposes? Finally, we will review the latest data regarding current and future therapeutics that target the adhesion process, describing their strength and weaknesses.

Autoimmunity is the failure of an organism to recognize its own constituent parts as self, which then allows an immune response against its own cells and tissues. Autoimmune diseases affect all organ systems and can be challenging to treat. Common examples include sarcoidosis, systemic lupus erythematosus, psoriasis, inflammatory bowel diseases, multiple sclerosis, and rheumatoid arthritis. In the USA up to 23.5 million people are affected by autoimmune diseases at any given time. The estimated annual treatment costs are greater than $100 billion. Most autoimmune diseases have a chronic or relapsing pattern, leading to a lifelong burden on patients, families, and society. Treatments for autoimmune diseases have traditionally been immunosuppressive and anti-inflammatory (steroids) drugs. Specific immunomodulatory biologic therapies, such as the TNF-α antagonists (e.g., etanercept), the B cell depleting agent rituximab, the anti-IL-6 receptor tocilizumab, and the costimulation blocker abatacept, have been shown to be useful in treating these conditions. However, some of these immunotherapies are associated with increased risk of adverse effects, such as susceptibility to infections, and additional therapeutic approaches are vastly needed.

Several mechanisms are thought to be responsible for the pathogenesis of autoimmune diseases. A normal immune response is expected to involve both B and T lymphocyte responses, as it requires the activation of B cells by T cells before the former can produce protective antibodies. Accordingly, any aberrant B or T cell signaling can lead to either loss of immune response or self-perpetuating auto-reactive immune response, which in turn will trigger inappropriate systemic lymphocyte activation and consequent decline in self-tolerance. Accordingly, aberrant signaling that results in inappropriate T-lymphocytes activation is the major cause of autoimmune diseases, thus targeting autoimmune T cells is a rational therapeutic objective.

There are multiple targetable steps involved in T-lymphocytes activation ranging from stimulation by the T-cell receptor (TCR), modulation by co-receptors, to migration to the site of injury. Since the pioneering work of Gowans in the 1960s, much progress has been made in understanding
The pivotal role of lymphocyte migration in autoimmunity. We now have considerable knowledge of the way in which lymphocytes are directed to distinct target tissues in immune responses and inflammation. Since T cells respond to pathogens only on direct contact with pathogen-derived antigens, they must migrate to sites where antigens are found. The TCR recognizes a peptide bound to major histocompatibility complex (MHC) on the surface of an antigen-presenting cell (APC). However, antigens occur in countless shapes and forms as there are multiple ways to generate an octapeptide (the length of peptide antigens held in the MHC binding groove). Our immune system copes with this diversity by generating a large army of ready-to-use T cells, each with a unique TCR. The repertoire of T cells that have never encountered an antigen, referred to as naive T cells, consists of around $1 \times 10^8$ distinct clones. However, the number of cells whose TCRs recognize any individual antigen is limited to several thousands.

This manuscript will discuss the migration of T cells, which are at the heart of many autoimmune responses. We will review the importance of increasing our comprehension of these events, focusing on migration, since they enclose a multitude of potential therapeutic targets for autoimmunity. We will address the following questions: What enables T cells to migrate to sites of injury, and what are the options to intervene? What is the contribution of co-receptors to T cell adhesion? How can we manipulate this knowledge for therapeutic purposes?

**Lymphocytes Homing: Interaction with Endothelial Cells**

Naive T cells must determine whether an antigen is present and whether it poses a threat to the body. Dendritic cells in secondary lymphoid organs, which collect and trap the antigen, provide this information. Naive T cells migrate to these organs, a process referred to as homing. An encounter with an antigen induces the proliferation of T-cell clones, yielding approximately 1,000 times more descendants with identical antigenic specificity. Next, these trigger lymphocytes acquire effector functions and migrate to sites of inflammation, where they interact with antigen-bearing parenchymal cells and other leukocytes, such as mast cells, macrophages, eosinophils, basophil, and neutrophils.

Specialized micro-vessels control the migration of T cells from the blood into tissues. In most cases, the micro vascular beds are the post capillary venules that interact efficiently with lymphocytes, thus avoiding the effects of cells adhesion on gas exchange in capillaries and on tissue perfusion in arterioles. Intravascular lymphocytes are subjected to extreme physical conditions. Flowing blood quickly dislodges cells that touch the vessel wall, because it exerts a shear stress force of up to approximately 50 dyn/cm². Such extreme fluid dynamics require T cells to use adhesion receptors, which form stable bonds with counter receptors in the vascular wall. Not only are adhesion receptors mechanical anchors, but they also function as tissue-specific recognition molecules. For example, the specialized endothelial cells that line the high endothelial venules in lymph nodes and Peyer’s patches constitutively express so-called addressins (mucosal vascular addressin cell adhesion molecule 1 [MAdCAM-1]), which support the homing of naive lymphocytes, whereas endothelial cells elsewhere permit no leukocyte binding unless they are exposed to inflammatory mediators. Thus, we distinguish two venular beds: those in lymphoid organs that recruit lymphocytes as a daily routine and those that solicit the entry of lymphocytes only when faced with inflammatory signals.

Leukocytes must engage several sequential adhesion pathways to leave the circulation. Initially, tethers are formed by adhesion receptors that are specialized to engage rapidly and with high tensile strength. The most important initiators of adhesion are the three selectins expressed on leukocytes (L-selectin), endothelial cells (E-selectin), and activated platelets (P-selectin). All selectins bind oligosaccharides related to sialyl-Lewis. The most relevant selectin-binding sugars are components of sialomucin-like glycoproteins.

Selectin-mediated bonds are too weak to arrest cells at the vessel wall. As the flowing blood exerts pressure, adhesion bonds dissociate at the cell’s upstream end, and new bonds form downstream. This results in a rolling motion that is much slower than that of free-flowing cells. To stop rolling, cells must engage additional adhesion molecules. These molecules belong to the integrin family, specifically leukocyte function associated antigen type 1 (LFA-1 or αLβ2 integrin) and very late antigen 4 (VLA-4 or α4β1). Integrins cause strong adhesion between the endothelium and its ligands. When adhesion by integrins occurs, the leukocyte is able to pass through the endothelium (transmigration) to the site of action. Integrins are transmembrane receptors that mediate the attachment between a cell and the tissues that surround it, such as other cells or the extracellular matrix. Integrins are not just “sticky” molecules. They have the capability to transmit information from outside the cell to inside the cell (outside-in signaling) and also have the ability to transmit the status of the cell to the ECM (inside-out signaling). These important signaling events allow the cell to communicate rapidly between both environments, intracellular, and extracellular. Therefore, in addition to transmitting mechanical forces across membranes, integrins are involved in cell signaling pathways associated with cell cycle, shape, and motility. There are many types of integrins, and different cells have a different selection on their surface.

Whereas selectins are constitutively active, integrins must be activated to mediate adhesion. Rolling T cells activate integrins when they receive signals from chemokines on endothelial surfaces. Chemokines are secreted polypeptides that bind to specific surface receptors, which transmit signals via G protein-couple receptors. Some chemokines trigger intravascular adhesion, whereas others direct the migration...
of leukocytes into and within the extravascular space. Secreted chemokines bind to heparin-like glycosaminoglycans on cell surfaces and in the extracellular matrix. Leukocytes can track down these immobilized chemokines. Since lymphocytes must be positioned correctly to interact with other cells, the pattern of chemokine receptors, the type, and distribution of chemokines in tissues critically influence immune responses. More than 50 chemokines have been identified. Such a large number of chemokines may direct lymphocytes to the anatomically distinct microenvironments where they need to function properly. Chemokines are classified as inflammatory or lymphoid. Inflammatory chemokines primarily attract neutrophils and monocytes. Their major sources are activated endothelial cells, epithelial cells, and leukocytes, when stimulated by endotoxins or inflammatory cytokines. Lymphoid chemokines are primarily produced in lymphoid tissues. They maintain the constitutive activity and compartmentalization of leukocytes in these organs.

As discussed above, homing of leukocytes involves three consecutive steps: tethering and rolling mediated by primary selectins, exposure to a chemotactic stimulus provided by chemokines and G-protein-coupled receptors, and arrest mediated by activated integrins. Each of these steps is necessary for lymphocytes to enter most tissues and for the accumulation at sites of inflammation. In leukocyte adhesion deficiency syndrome (LAD), a genetic defect either in β2 integrins (type I) or in fucosylated selectin ligands (type II), leukocytes cannot stop or roll, respectively; this syndrome is characterized by marked leukocytosis and frequent bacterial infections. The pronounced lymphocytosis in patients with Bordetella pertussis infection is caused by pertussis toxin, which inactivates the signaling of G proteins and blocks chemokine-mediated activation of integrins. Hence, lymphocytes cannot stop rolling, and they remain in the circulation.

The Immunological Synapse: Interaction with Antigen Presenting Cells

Homing to any lymphoid organs remains irrelevant unless lymphocytes encounter antigens in the appropriate context. Subgroups of monocytes are thought to home to tissues and differentiate into immature dendritic cells. These cells express receptors for inflammatory chemokines and chemoattractants that are released during infections. This ability enables them to enter and migrate through inflamed tissues. Immature dendritic cells patrol tissues and engulf microorganisms, dead cells, and cellular debris. On exposure to inflammatory stimuli, they travel to local lymph nodes through afferent lymph vessels, undergo further maturation, lose their receptors for inflammatory chemokines, and up-regulate the expression of receptors for lymphoid chemokines. These changes allow dendritic cells to find their way to the T-cell area of lymph nodes. While in transit, dendritic cells also ready their apparatus for antigen presentation and begin to produce chemokines that make them attractive to T cells awaiting their arrival in lymph nodes.

The immunological synapse (IS) is the interface between an APC and a lymphocyte. Abraham Kupfer first discovered it in 1995, when he showed three-dimensional images of immune cells interacting with one another. This is the junction between a T cell and an APC, and it consists of a central cluster of TCRs surrounded by a ring of adhesion molecules. Key molecules in the synapse are the TCR and its counterpart the MHC. Also important are LFA-1, ICAM-1, CD28, and CD80/CD86. The immune synapse is also known as the supramolecular activation cluster or SMAC. This structure is composed of concentric rings each containing a peculiar mix of molecules: c-SMAC (central-SMAC) composed of θ isoform of protein kinase C, CD2, CD4, CD8, CD28, Lck, and Fyn; p-SMAC (peripheral-SMAC) composed of the lymphocyte function-associated antigen-1 (LFA-1), CTLA-4, and the cytoskeletal protein talin; d-SMAC (distal-SMAC) composed of CD43 and CD45 molecules. The IS formation has been shown to be an active and dynamic mechanism that allows T cells to distinguish potential antigenic ligands.

The Effector Cells

During primary responses, T cells differentiate into effector cells in lymphoid organs. They must immediately migrate to peripheral tissues that contain pathogens, which elicit local inflammation by stimulating innate immune cells. Thus, effector cells up-regulate the expression of receptors for inflammation-induced endothelial adhesion molecules and inflammatory chemokines.

Different pathogens elicit different effector responses mediated by either Th1 or Th2 cells. Th1 and Th2 cells express distinct receptors and obey different traffic signals. Distinctive chemokine receptors on Th1 cells include CCR5 and CXCR3 that bind inflammatory chemokines. In patients with rheumatoid arthritis and multiple sclerosis (Th1-related diseases), virtually all infiltrating T cells express CCR5 and CXCR3. Individuals with a homozygous mutation that disrupts the CCR5 gene may also be less susceptible to some inflammatory disorders, including rheumatoid arthritis. Adhesion molecules also have a role; Th1 cells express selectin ligands abundantly. P- and E-selectin, which occur on inflamed endothelium, and their ligand, P-selectin glycoprotein ligand 1, are critical for the migration of Th1 cells to inflamed skin and peritoneum. The expression of fucosyltransferase VII is necessary for cells to synthesize selectin ligands. This enzyme is induced by interleukin-12, which drives the differentiation of Th1 cells. The characteristic chemokine receptor on Th2 cells is CCR3, the eotaxin receptor. Eotaxin is involved in the recruitment of eosinophils into hyper-reactive airways and is prominent in mucosal tissues where allergic and antiparasitic responses are occurring. The production of eotaxin is stimulated by cytokines secreted by Th2 cells, such as IL-4 and IL-13. Other chemoattractant receptors are also preferentially expressed on Th2 cells, including CCR4, CCR8, and CXCR4.
The traffic signals that direct CD8+ effector cells to inflamed tissues have not been studied as extensively as those for the CD4+ subgroup, but they appear to be similar. When stimulated by antigen, cytotoxic T cells secrete inflammatory chemokines. Through this mechanism, CD8+ T cells are thought to increase the recruitment of neutrophils, monocytes, and Th1 cells.

Antigen-triggered T cells often display tissue specificity that may improve their chances of reencountering an antigen. T cells that have been exposed to cutaneous pathogens in skin-draining lymph nodes migrate preferentially to the skin, whereas cells that arise in Peyer’s patches in response to entero viral infections are most useful in the gut. Indeed, lymphocytes express different homing receptors after stimulation by the same antigen, depending on whether it is given orally or parenterally. The best-understood tissue-selective homing pathways are in the skin and intestines, but there may be other selective migration mediators, such as to the lungs, joints, and central nervous system.

Costimulation and Lymphocyte Adhesion

T cells require at least two signals to become fully activated. An initial signal, which is antigen-specific, delivered through the TCR that interacts with peptide-MHC molecules on the membrane of APC. A second signal, the costimulatory signal, is antigen nonspecific and is provided by the interaction between costimulatory molecules expressed on the membrane of the APC and the T cell. One of the most characterized costimulatory molecules expressed by T cells is CD28, which interacts with CD80 (B7.1) and CD86 (B7.2) on the membrane of APC. T cell costimulation is necessary for proliferation, differentiation, and survival. Activation of T cells without costimulation can lead to T cell anergy, T cell deletion, or development of immune tolerance. Another co-receptor expressed by T cells is Cytotoxic T Lymphocyte Antigen-4 (CTLA-4), which interacts with the same ligand on the APC. T cell costimulation may have opposing effects. While the costimulation pathway initiated by the CD28 receptor has an activating effect on naïve T cells, the pathway initiated by CTLA-4 has an inhibitory effect on T-cell activation. Interestingly, CTLA-4 signaling is not associated exclusively with inhibitory effects.

We were able to show very clearly that CTLA-4 signaling is associated with increased adhesion to ICAM-1 and is important for IS stabilization. Thus, CTLA-4 should be regarded as a modulator.

These co-modulation pathways have been found to be associated with autoimmune diseases in humans. Mutations in the CTLA-4 gene have been associated with insulin-dependent diabetes mellitus, Graves’ disease, Hashimoto’s thyroiditis, celiac disease, systemic lupus erythematosus, and primary biliary cirrhosis. Polymorphisms of the same gene are also associated with autoimmune diseases, such as autoimmune thyroid disease and multiple sclerosis, though this association is often weak. In systemic lupus erythematosus, for example, the splice variant CTLA-4 is aberrantly produced and is found in the serum of patients with active disease. Greater understanding of these pathways is still needed as it holds promising targets for future therapies as there are multiple intertwined effects within it that could be manipulated in order to fight autoimmune diseases.

Fusion protein of CTLA-4 (CTLA-4-Ig) has been approved by the FDA for rheumatoid arthritis. The fusion protein CTLA4-Ig is commercially available as Orencia (abatacept). Orencia works by binding to CD80 and CD86 on the APC, thus preventing interactions with CD28 expressed on the T cells. It is indicated for patients with moderate to severe rheumatoid arthritis, who have had minimal response to other DMARDs (i.e., Methotrexate) and biologics (i.e., Etanercept). Orencia has undergone multiple randomized, double blind placebo-controlled trials. Two of the larger trials were the ATTAIN trial (Abatacept Trial in Treatment of Anti-TNF Inadequate responders) and AIM trial (Abatacept in Inadequate responders to Methotrexate). These studies led to Orencia’s FDA approval in 2005. When compared to placebo, Orencia consistently showed improvements in American College of Rheumatology (ACR) symptom severity scores. ACR criteria is a measured reduction in tenderness and joint swelling and improvement in three of the following: 1. the patient’s overall assessment of his or her own symptoms, 2. the physician’s global assessment of the patient’s symptoms, 3. the patient’s assessment of his or her own pain, 4. the patient’s assessment of his or her own physical functioning, and 5. the results of an erythrocyte sedimentation rate or C-reactive protein. The response rates are then further divided into the percent of patients who had a 20%, 50%, and 70% (ACR20, ACR50, ACR70) improvement in tender and swollen joints and three of the five above criteria. Interestingly, Orencia is also showing promising outcomes in psoriatic arthritis and type 1 autoimmune diabetes. A randomized, placebo-controlled study in type 1 diabetics showed that Orencia could delay c-peptide reduction by about 10 months. A second-generation form of CTLA-4-Ig known as belatacept was recently approved by the FDA based on favorable results from a randomized trial in renal transplantation. Belatacept works similar to Orencia by binding to CD80 and CD86, therefore preventing T-cell costimulation by CD28. In two studies where belatacept was compared to a cyclosporine regimen in renal transplant recipients, Belatacept was shown to be just as effective by biopsy and also showed a better side effects profile with lower blood pressure and lipid panels. Belatacept also presented better maintenance of GFR compared to cyclosporine at 2 years.

**Direct Targeting of the Adhesion Process**

Since chemokine receptors and adhesion molecules are promising targets for anti-inflammatory therapies, the development of antagonists is among the most actively pursued areas in pharmaceutical research. Several landmark studies
are worth mentioning. Two studies reported the profound effect of antagonists of Th2 chemokines and α4β1 integrins in animal models of asthma. Antibodies to α4 integrins also block the development of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. Numerous antibodies, recombinant soluble adhesion molecules, receptor-blocking mutant chemokines, and small molecules are being evaluated as treatments for asthma, multiple sclerosis, inflammatory bowel disease, arthritis, and psoriasis, and some should work. Small molecules are the agents of choice for commercial development, and there are already several potent small-molecule antagonists of chemokine receptors. Interactions involving integrins or selectins are more difficult to inhibit with small molecules, but there has been successes with antagonists of interactions between α4β1 integrin and vascular-cell adhesion molecule 1 (VCAM-1) and between LFA-1 and intercellular adhesion molecule 1. These advances should enable clinicians to choose between highly selective treatments. For example, by modulating essential homing elements of naive T cells or dendritic cells, clinicians might prevent, attenuate, or enhance immune responses to new antigens, such as allografts or vaccines. Because this treatment would not interfere with the responses of memory T cells, it should not be globally immunosuppressive. Similarly, new drugs that inhibit organ-specific homing cascades of populations of pathogenic leukocytes would permit the use of tissue-selective anti-inflammatory interventions.

Since selectins play a crucial role in the first step of leukocyte trafficking, they appear to be promising targets for future therapeutic treatments in autoimmune diseases. Patients with leukocyte adhesion deficiency (LAD) type II lack selectins and as a result are susceptible to multiple severe infections. Unfortunately to date, therapies targeted at selectins have not been successful, a fact attributed to the redundancy of leukocyte trafficking. The ability of leukocytes to roll via VLA-4 integrins plays as an example of such trafficking.

Due to their importance in recruiting inflammatory cells to sites of inflammation, interfering with integrins function specifically could serve as a target for autoimmunity. To date, integrins have been the most successful therapeutic targets as will be described below. Multiple clinical trials have targeted integrins. Significant achievements have been accomplished in psoriasis. Efalizumab is a monoclonal antibody against CD11a. Efalizumab is FDA approved to treat psoriasis. Efalizumab was compared to placebo in a randomized, double blind study and showed improvement in Psoriasis Area and Severity Index (PASI-75) of 39% and 27% in high and low dose groups respectively; placebo only had a 2% improvement at 12 weeks. At 12 weeks the treatment group was again randomized to placebo or efalizumab. The efalizumab group showed improvement in remission through week 24 at a rate of 77%, whereas the placebo group only had a remission rate of 20%. Unfortunately, in 2009, efalizumab was voluntarily removed from the market, due to concern that it may be associated with progressive multifocal leukoencephalopathy in patients treated for over 3 years. Efalizumab was efficacious in treating psoriasis, and it is assumed that key mechanisms of action include reduced migration of T cells into the skin, as well as reduced T-cell activation through interference with immune synapse formation between T cells and APC. In T-cell activation experiments from psoriasis patients, T-cell activation was also reduced, possibly reflecting interference with immune synapse formation. In a recent work, it was demonstrated that reduced T-cell responsiveness independent of immune synapse formation (suggesting a direct effect on T cell) was achieved with this agent.

Promising results have also been achieved in the treatment of Crohn’s disease with natalizumab, which is a humanized mAb to the α4 subunits, which shows superior response and remission rates when compared to placebo. Natalizumab is used in moderate to severe Crohn’s disease when patients do not benefit or cannot tolerate conventional treatment, such as steroids and anti-TNF-α. One study (ENACT-2) that led to FDA approval showed that patients who displayed an initial response with natalizumab were more likely to benefit from increased remission rates. The other study (ENCORE) that led to FDA approval showed that natalizumab led to responses in 60% of patients compared to 44% in the placebo group. Natalizumab can also be used to treat relapsing remitting multiple sclerosis. Two trials led to FDA approval in 2004. First, the AFFIRM trial, a large double blind, randomized trial that compared natalizumab versus placebo for 2 years, showed a relative risk reduction of relapse at 1 year of 68% (p < 0.001); these results were maintained at 2 years. Second, the SENTINEL study compared natalizumab or placebo added to interferon-β. The results were similar to the AFFIRM study. There is, however, concern that natalizumab may increase risk for PML. In 2005, it was briefly pulled from the market but returned in 2006 with some limitations (all patients and practitioners using natalizumab must take part in special education classes).

Of note is another drug that has shown promise in treating Crohn’s disease, which does not have concern for PML, and is currently in phase III trials. Vedolizumab is an α4-β7 integrin inhibitor; this allows normal migration of lymphocytes to all organs but the gut and gut associated lymphoid tissue (GALT). In the study GEMINI 1, 895 patients who had failed previous treatments with corticosteroids, thiopurines, or anti-tumor factor agents were randomized to vedolizumab every 4 weeks, vedolizumab every 8 weeks, or placebo. After 52 weeks, the 4 week vedolizumab group had clinical remission at 44.8%, and the 8 week group had a rate of 41.8%. The placebo group had a remission rate of 15.9%. As for side effects, such as infection, all groups had similar rates. It is important to note that natalizumab and vedolizumab both inhibit α4-β7, but with natalizumab, there is a concern for
PML, whereas with the more selective vedolizumab there is not. The hope for future therapeutics is to be selective for a specific target tissue as to avoid severe immune suppression.

**Future Directions**

Many intriguing questions remain. What are the additional adhesion molecules employed during migration of lymphocytes to tissues? How do lymphocytes decide which signal to follow when faced with different chemoattractants? How do they decide when to stay and when to leave? What orchestrates the transportation, presentation, and neutralization of chemokines? Are there negative modulators of migration, such as anti-adhesins or repellents, in addition to chemoattractants and adhesion molecules?

**Disclosure Statement**

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