T-LYMPHOCYTE INTERACTIONS WITH ENDOTHELIUM AND EXTRACELLULAR MATRIX

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ABSTRACT: T-lymphocyte movement out of the bloodstream and into tissue is critical to the success of these cells in their role in immunosurveillance. This process involves interactions of the T-cell with endothelium as well as with extracellular matrix. Central to these interactions are a number of T-cell adhesion molecules and their endothelial and extracellular matrix ligands. The identification and functional characterization of adhesion molecules have been the subject of intensive research in recent years. We highlight here the latest developments in this rapidly expanding field as they pertain to T-cell interactions with endothelial cells and extracellular matrix components, including: (1) identification of adhesion molecule families, including the selectins, mucins, integrins, immunoglobulin superfamily members, and cadherins; (2) elucidation of the multi-step adhesion cascade that mediates the rolling, arrest, and eventual diapedesis of T-cells through the vascular endothelium into the surrounding tissue; (3) the changes in adhesion molecule expression that accompany T-cell maturation and activation, and the impact of those changes on T-cell migration; (4) the functional relevance of the extracellular matrix for T-cell function; and (5) the clinical relevance of adhesion molecules and the potential for targeting these molecules for the amelioration of immune-mediated diseases.

Key words. Cell adhesion, integrin, selectin, extracellular matrix, endothelium.

(I) *Introduction*

Adhesion receptors allow circulating leukocytes to initiate physical contact with the extracellular environment and to respond appropriately to information found in that environment. For T-lymphocytes, this requirement for adhesion can be broken down conceptually into two components. First, T-cells respond to foreign antigen only when peptides derived from that antigen are associated with an appropriate major histocompatibility (MHC) antigen and are recognized by the antigen-specific T-cell receptor. Therefore, T-cell function requires the physical interaction of T-cells with antigen-presenting cells. Adhesion receptors such as the lymphocyte function-associated antigen-1 (LFA-1) integrin and the CD2 antigen, as well as others, have been shown to play essential roles in T-cell interactions with antigen-presenting cells. Second, T-cells continuously circulate throughout the body in order to maximize the chances that the rare T-cell expressing a T-cell receptor with the appropriate specificity encounters the peptide/MHC combination that leads to the signal transduction events that result in T-cell activation, proliferation, and effector functions. Changes in adhesion molecule expression that occur as a result of an encounter with antigen permanently alter the recirculation pattern of the resulting memory T-cells. Furthermore, the process of inflammation results in the active and specific recruitment of T-cells and other leukocytes into the inflammatory site.

The critical cell-cell interaction mediating the specificity of T-cell recirculation and recruitment is the adhesion of circulating T-cells with vascular endothelial cells (EC). Recent advances in our understanding of this adhesive interaction have illustrated the sequential involvement of different adhesion molecules as well as a critical activation step, providing for considerable complexity and regulatory flexibility. Although not as extensively appreciated, components of the extracellular matrix (ECM) also play a vital role in both T-cell recognition of antigen and T-cell recirculation and recruitment. This review highlights recent insights into lymphocyte interactions with endothelium and ECM, with a specific

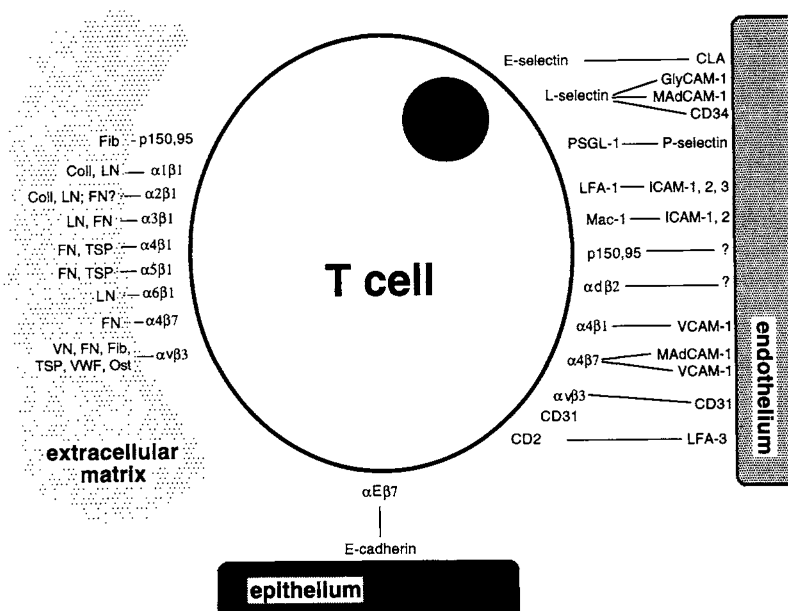

Figure 1. Adhesion molecules and their ligands. Known receptor-ligand interactions mediating T-cell adhesion to endothelium, epithelium, and extracellular matrix are indicated. This figure illustrates the vast number of adhesion molecules utilized by T-cells, but is not meant to indicate that any specific T-cell expresses and/or utilizes all of these adhesion receptors. FN, fibronectin; Fib, fibrinogen; Coll, collagen; LN, laminin; VN, vitronectin; TSP, thrombospondin; VWF, von Willebrand's factor; Ost, osteopontin.

emphasis on T-lymphocytes. We have attempted to focus on: (1) novel adhesion molecules involved in T-cell interactions with endothelium and ECM; (2) the dynamics of the adhesion cascade mediating T-cell adhesion to ECs; (3) changes in adhesion molecule expression upon peripheral T-cell differentiation; and (4) the concept that the ECM represents a rich milieu of information to which the T-cell responds during both T-cell migration and recognition of antigen. We regret that space constraints prevent us from adequately citing all of the seminal contributions by investigators in this field. In most of the sections that follow, we have cited many other outstanding reviews that have treated these specific areas in more detail than was possible here.

(II) Families of Adhesion Molecules

There are five structural families of adhesion molecules that have been described to date: the selectins, the mucins, the integrins, the immunoglobulin superfamily (IgSF), and the cadherins. These families are utilized by T-cells to interact with endothelium, epithelium, and ECM components (Fig. 1).

(A) Selectins

The selectins are a group of cell-surface glycoproteins found on endothelium, platelets, and leukocytes (reviewed in Carlos and Harlan, 1994; Lasky, 1995; Tedder *et al.*, 1995). The selectins are the only family of structural adhesion molecules whose single known purpose is to mediate leukocyte-EC interactions. The selectins are important in several aspects of lymphocyte-endothelial interactions, including the initial intravascular arrest of circulating lymphocytes and subsequent rolling on intravascular ECs. The three-member family includes E-selectin (CD62E, ELAM-1), P-selectin (CD62P, GMP-140), and L-selectin (CD62L). Similar in structure, the selectins are made up of an N-terminal C-type lectin-like domain, an epidermal-growth-factor-like domain, a variable number of consensus sequences (similar to complement-regulatory proteins), a membrane spanning sequence, and a short cytoplasmic tail.

Although structurally similar, the three selectin family members show differences with respect to their tissue distribution and regulation (reviewed in Carlos and Harlan, 1994). E-selectin is expressed primarily on activated EC and binds to ligands expressed on neutrophils, monocytes, basophils, and a subset of $CD4^+$ memory T-cells. E-selectin is transcriptionally up-regulated by multiple stimuli, including interleukin (IL)-1, tumor necrosis factor (TNF)-α, and lipopolysaccharide (LPS) (Carlos and Harlan, 1994). P-selectin is expressed on platelets and activated endothelium, and binds ligands expressed on neutrophils, monocytes, and a minority of $CD4^+$ memory T-cells. P-selectin is stored and synthesized in granules (α granules in platelets and Weibel-Palade bodies in EC), and up-regulation can occur in minutes after exposure to many stimuli, including thrombin, histamine, and cytokines (Carlos and Harlan, 1994). L-selectin was initially identified as being important in lymphocyte binding to high endothelial venules (HEV), which are specialized postcapillary venules of lymphoid tissue. When the Mel-14 monoclonal antibody (mAb) was used in mice, L-selectin was implicated as a homing receptor involved in lymphocyte migration into peripheral lymph nodes (PLN) (reviewed in Lasky, 1995). Subsequently, L-selectin was found to be expressed on most peripheral blood leukocytes. Unlike the other selectins, L-selectin is constitutively expressed on the cell surface, and can be shed after activation by protease cleavage near the membrane insertion site (Lasky, 1995; Tedder *et al.*, 1995). L-selectin cleavage has been speculated to allow for lymphocyte detachment and possible attenuation of the immune response. In support of this hypothesis, soluble L-selectin *in vitro* has been shown partially to inhibit leukocyte adhesion to cytokine-stimulated endothelium (McEver, 1994).

To date, several heavily glycosylated proteins containing O-linked carbohydrate side-chains have been identified as physiologic ligands for the selectins, including glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1, Sgp50), CD34 (Sgp90), mucosal-addressin

cell adhesion molecule-1 (MAdCAM-1), P-selectin glycoprotein ligand-1 (PSGL-1), and cutaneous lymphocyte antigen (CLA) (see below). Selectins bind simple carbohydrate moieties on their ligands *via* a calcium-dependent interaction with the lectin domain in a unique protein-nonprotein interaction. One of the first selectin carbohydrate ligands to be identified was the small fucosylated tetrasaccharide, sialyl Lewis X (SLe^x). SLe^x [NeuNacα2,3Galα1,4(Fucα1,3)GlcNAc, where Nac = sialic acid, Gal = galactose, Fuc = fucose, and GlcNAc = N acetyl glucosamine] is thought to be the minimal oligosaccharide needed for selectin-mediated adhesion. SLe^x binds all three selectins with low, but differing, affinities (McEver, 1994; Lasky, 1995). Nonetheless, SLe^x has been shown to mediate lymphocyte adhesion to HEV in frozen sections of lymph node (LN) tissue by means of the Stamper-Woodruff HEV binding assay. The relevance of selectins to leukocyte interactions with endothelium is illustrated by the recent identification of a clinical syndrome, leukocyte adhesion deficiency type II (LAD type II). Patients with LAD type II suffer from a generalized defect in fucose metabolism that results in a lack of SLe^x expression. Consequently, neutrophils from LAD type II patients fail to bind either E-selectin or P-selectin expressed on endothelial cells (Price *et al.*, 1994). The clinical manifestations of LAD type II are similar to those described for LAD (see below).

(B) Mucins

Mucins are glycoproteins containing primarily O-linked carbohydrate side-chains attached to serine or threonine residues (reviewed in Shimizu and Shaw, 1993; Lasky, 1995). Mucins are one of the newest families of adhesion molecules to be identified, and there is considerable speculation that many family members are yet to be found. Three mucins have been identified as selectin ligands: GlyCAM-1, CD34, and MAdCAM-1.

GlyCAM-1—a mucin-like glycoprotein with two sulfated, sialylated, and fucosylated heavily O-linked oligosaccharide chains—is expressed on PLN HEV and hematopoietic progenitors, and binds L-selectin. GlyCAM-1 lacks a conventional transmembrane region and is uniquely anchored to the EC surface by an incompletely defined mechanism. It is thought that GlyCAM-1 is shed as a potentially soluble protein (Shimizu and Shaw, 1993), although the physiological significance of this shed molecule is not yet understood. Recently, a sulfated SLe^x-like core of GlyCAM-1, (SO_4-6)Galβ1-4GlcNAc, was identified and may serve as the selectin recognition determinant on GlyCAM-1 (Hemmerich *et al.*, 1994).

CD34 is a sialomucin-like protein ligand for L-selectin that is widely expressed intravascularly. Using an anti-CD34 mAb, Baumhueter *et al.* (1994) demonstrated intravascular staining in all lymphoid and non-lymphoid organs in mice. Although CD34 expression was maintained at sites of inflammation, no changes in expression levels were noted immunohistologically. Based on the ubiquitous expression of CD34, even in mature PLN, where L-selectin-mediated lymphocyte-endothelial binding does not occur, the authors speculated that changes in CD34-mediated binding to L-selectin are regulated by differential glycosylation of the carbohydrate moieties (Baumhueter *et al.*, 1994).

MAdCAM-1 is a 58-66-kDa protein initially identified as the mucosal addressin recognized by the mAb MECA-367 (reviewed in Carlos and Harlan, 1994). MAdCAM-1 is expressed primarily on Peyer's patch HEV, mesenteric LN HEV, and mucosal lamina propria venules (Picker, 1994). MAdCAM-1 is a novel protein containing both a mucin-like domain, that is capable of binding L-selectin, and an IgSF-like domain, that is capable of binding $\alpha4\beta7$ (Berg *et al.*, 1993). Thus, MAdCAM-1 can support both lymphocyte rolling and integrin-mediated adhesion.

Additional members of the mucin family that are expressed on lymphocytes and are important in lymphocyte-endothelial interactions include the P-selectin glycoprotein ligand, PSGL-1, and the E-selectin ligand, CLA (Shimizu and Shaw, 1993; Picker, 1994). PSGL-1 is found on the surfaces of myeloid cells and activated lymphocytes. CLA is selectively expressed on memory T-cells that migrate to the skin (see below). Both of these molecules contain a dense network of O-linked carbohydrate chains. These long side-chains are predicted to bear a heavy negative charge, but are long enough to help the ligand gain exposure above the glycocalyx of the cell membrane. Thus, only in specific cell-cell interactions, where the apposing cell has the specific selectin-ligand receptors, will these negative charges be overcome and adhesion ensue (Shimizu and Shaw, 1993).

(C) Integrins

The integrins are a large family of cell-surface proteins, expressed as $\alpha\beta$ heterodimers, that mediate cell-cell and cell-ECM interactions (reviewed in Carlos and Harlan, 1994; Diamond and Springer, 1994). The integrins are important in the regulation of many cellular functions, including embryonic development, tumor invasion and metastasis, wound healing, and the immune response. There have been 15 α chains and 8 β chains identified to date, giving rise to at least 21 different noncovalently bound $\alpha\beta$ combinations. The α subunits, which vary in size from 120-180 kDa, and the β subunits, which vary in size from 90-110 kDa, are transmembrane proteins with generally short cytoplasmic domains. The exception is $\beta4$, which has a 1000-amino-acid-long cytoplasmic tail. The cytoplasmic tails are important in intracellular signaling and, at least for the β subunits, are important for interacting with components of the cytoskeleton, such as α-actinin and talin (Clark and Brugge, 1995). The specificity of the integrin receptor is due to the particular $\alpha\beta$

combination that forms the extracellular globular head. An individual integrin can have several different ligands. For example, α4β1 [very late antigen (VLA) -4] can bind fibronectin (FN), invasin, and vascular cell adhesion molecule-1 (VCAM-1). In addition, a given ligand can be recognized by several different integrins. FN is recognized by α3β1 (VLA-3), α4β1, α5β1 (VLA-5), α4β7, and αvβ3 (Diamond and Springer, 1994). With the exception of mature erythrocytes, at least one integrin receptor is expressed on all cell types (Diamond and Springer, 1994). Integrin ligands include members of the IgSF [intercellular adhesion molecule (ICAM)-1, ICAM-2, VCAM-1], ECM components (see below), and viral and bacterial proteins. Important integrin receptor-ligand pairs in lymphocyte-endothelial interactions include: the LFA-1/ICAMs -1 and -2, α4β1/VCAM-1, α4β7/VCAM-1, and α4β7/MAdCAM-1.

Several recently described integrin molecules, pertinent to lymphocyte-endothelial interactions, deserve attention. Until recently, the β2 integrin family was made up of three structurally related β2-containing heterodimers: LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), and p150,95 (CD11c/CD18). A novel β2 integrin, αdβ2, has been discovered on the cell surface of canine leukocytes (Danilenko *et al.*, 1995). The 155-kDa αd chain is expressed as a heterodimer with β2 on macrophage subpopulations, and two types of peripheral blood $CD8^+$ lymphocytes, large granular lymphocytes and small lymphocytes (Danilenko *et al.*, 1995). The amino acid sequence of canine αdβ2 bears significant homology to the recently identified human αdβ2 integrin obtained by cDNA cloning (cited in Danilenko *et al.*, 1995). The ligands and functions attributable to canine αdβ2 await further evaluation, but human αdβ2 has been reported to bind preferentially to ICAM-3 over ICAM-1 (cited in Danilenko *et al.*, 1995).

The αvβ3 integrin, previously known only to bind many ECM proteins and components of the coagulation-cascade, has recently been shown to be a ligand for the IgSF member, CD31 [platelet-endothelial cell adhesion molecule (PECAM)-1] (Piali *et al.*, 1995). This is the first heterophilic CD31 ligand to be identified. The αvβ3:CD31 interaction is cation-dependent, involves the second Ig-like domain of CD31, and can be blocked by anti-CD31 and anti-βvβ3 antibodies (Piali *et al.*, 1995). αvβ3 is expressed by many different cell types, including ECs, mast cells, natural killer (NK) cells, dendritic epidermal T-cells, and activated T-cells (Piali *et al.*, 1995). The ability of αvβ3- and CD31-specific mAbs partially to block the adhesion of lymphokine-activated killer cells to an endothelial cell line suggests that the αvβ3:CD31 interaction may play a role in lymphocyte adhesion to endothelium *in vivo*.

Several other recent discoveries have involved the α4 integrins and their ligands. By protein isolation, amino acid sequence analysis, and peptide blocking, it was shown that α4β1 and α4β7 recognize a leucine-aspartic acid-valine (LDV) sequence found in the α4 subunit itself (Altevogt *et al.*, 1995). This result is consistent with earlier findings demonstrating inhibitory or enhancing effects of α4-specific mAbs on cell-cell interactions in lymphoid cell populations that do not express any of the previously identified α4 ligands. In another study, the crystal structure of a α4β1-binding fragment of the first two domains of the VCAM-1 molecule was determined. This model demonstrated a highly-exposed integrin-binding motif (Q^{38}IDSPL) contained within the N-terminal region of the loop between β-strands C and D in domain 1 of the VCAM-1 molecule (Jones *et al.*, 1995). This binding motif exhibited a unique conformation that the authors predicted will be common to all integrin-binding IgSF motifs. Last, a study looking at the CS-1 region of FN, VCAM-1, and MAdCAM-1 binding sites on α4β1 found that the putative binding sites were all contained within a small region of the α4 chain (residues 107-268) (Kamata *et al.*, 1995).

A great deal of our insight into the regulation and function of the β2 integrins has been elucidated by a severe genetic immunodeficiency syndrome, leukocyte adhesion deficiency (LAD), that was first identified in 1979 among six infants with delayed separation of the umbilical cord, recurrent bacterial infections, and abnormal neutrophil function (reviewed in Harlan, 1993). The major clinical manifestations of the LAD syndrome include recurrent bacterial soft tissue infections, chronic gingivoperiodontitis, and impaired wound healing, all in the face of a chronic neutrophilic leukocytosis. The LAD syndrome is now known to be due to various mutations in the β2 subunit gene (Harlan, 1993; Lopez Rodriguez *et al.*, 1993). The LAD phenotype can range from severe, which is associated with death during infancy and $< 1\%$ of the normal cell-surface LFA-1, Mac-1, and p150,95 integrins, to mild, which is associated with fewer infections and survival into adulthood, and up to 30% of normal cell-surface β2 integrin expression (Harlan, 1993). Although neutrophil emigration is profoundly impaired in LAD patients, lymphocyte trafficking is less severely affected. T-lymphocytes from LAD patients demonstrate reduced levels of basal binding to unstimulated endothelium, but demonstrate a normal enhancement of adhesion to TNF-α-stimulated endothelium, due to α4β1-mediated interactions (Vennegoor *et al.*, 1992).

(D) Immunoglobulin Superfamily

The IgSF encodes a large family of proteins with many different functions, including antigen recognition and cellular adhesion (reviewed in Picker, 1994; Carlos and Harlan, 1994; Hynes, 1994). Members of this family all contain at least one region showing similarity to a com-

mon, well-preserved ancestral protein domain originally identified in immunoglobulin variable and constant regions. These domains (70-110 amino acids) are arranged in a sandwich of two sheets of anti-parallel β strands apposed *via* hydrophobic faces. These β strand loops serve as the specific binding sites for the IgSF counter-receptors. Several members of the IgSF are involved in T-cell-EC interactions, namely, CD31 on T-cells and ICAM-1 (CD54), ICAM-2 (CD102), VCAM-1 (CD106), CD31, and MAdCAM-1 on EC.

ICAM-1 is expressed on EC, thymic and mucosal epithelial cells, mononuclear cells, and fibroblasts. ICAM-1 has five immunoglobulin-like domains and is recognized by LFA-1 and Mac-1. Similar to E-selectin, ICAM-1 can be transcriptionally up-regulated by TNFβ, IL-1, and LPS, but has a longer time-course of expression (reviewed in Carlos and Harlan, 1994). ICAM-2 has two Ig-like domains and is recognized by LFA-1. ICAM-2, like ICAM-1, is expressed on resting ECs but, unlike ICAM-1, is not up-regulated by inflammatory stimuli (Carlos and Harlan, 1994).

VCAM-1 is not expressed on normal endothelium, but can be transcriptionally up-regulated by the same inflammatory stimuli listed above for E-selectin and ICAM-1. Although the predominant form of VCAM-1 on endothelium consists of 7 Ig domains, VCAM-1 forms containing 6 and 8 domains have also been identified (Hession *et al.*, 1991). The 6-domain form is generated through alternative splicing and lacks the fourth domain. In addition, a phosphatidyl inositol (PI)-linked form, containing only the 3 N-terminal Ig-like domains, has been identified on inflamed murine tissues (Moy *et al.*, 1993). $\alpha 4\beta 1$ is capable of interacting with all forms of VCAM-1, including the PI-linked form, and is known to interact with domains 1 and 4 on the 7-domain form of VCAM-1 (Kilger *et al.*, 1995).$\alpha 4\beta 7$ binds the 7-domain form of VCAM-1; other forms have not been tested.

Recent additions to the IgSF include ICAM-3, CD31, and MAdCAM-1. Unlike ICAM-1 and ICAM-2, ICAM-3 is not expressed on EC, but is expressed at high levels on T-lymphocytes (De Fougerolles and Springer, 1992; Campanero *et al.*, 1994). ICAM-3 participates in T-cell activation and can regulate the LFA-1/ICAM-1 adhesion pathway (Campanero *et al.*, 1994). CD31 is constitutively expressed on HEV, platelets, neutrophils, monocytes, and on certain T-cell subsets. CD31 is known to mediate cell-cell interactions *via* a homophilic interaction. Expression of CD31 is primarily localized to intercellular contact points, where CD31-CD31 homophilic binding occurs. As described above, CD31 can also interact with the $\alpha v\beta 3$ integrin. In addition to its role as an adhesion molecule, engagement of the CD31 receptor can transduce intracellular signals that result in up-regulation of $\alpha 4$ integrin activity (Tanaka *et al.*, 1992). MAdCAM-1 is expressed primarily on Peyer's patch HEV, mesenteric LN HEV, and mucosal lamina propria venules and is the only IgSF member known to date to have both mucin-like and IgSF-like characteristics.

(E) Cadherins

The cadherins are a family of calcium-dependent transmembrane cell adhesion proteins that bind primarily through homophilic and heterophilic interactions (reviewed in Shapiro *et al.*, 1995; Patel and Gumbiner, 1995). Cadherins are known to be important in embryonic development and maintenance of tissue architecture *via* cell-cell junctions, including desmosomes and zonula adherens. New evidence suggests a possible role for cadherins in lymphocyte localization and immune surveillance (Cepek *et al.*, 1994). All cadherins cloned to date have a similar primary structure. These proteins are 723-748 amino acids long, with a putative signal peptide sequence, an extracellular portion containing 3-5 tandem repeat domains, a hydrophobic transmembrane anchor, and a long cytoplasmic tail.

There are currently four subclasses of cadherins: (1) the E-cadherins (uvomorulin, cell CAM 120/80, Arc-1 or L-CAM), which are expressed on adult epithelial cells; (2) the N-cadherins (A-CAM, N-Cal-CAM), which are expressed on adult neural tissue, the lens of the eye, and muscle; (3) the P-cadherins, which are transiently expressed in several tissues during development as well as in the placenta and epithelium in adults; and (4) the R-cadherins. Studies using N-cadherin have demonstrated that both the extracellular domain and the cytoplasmic tail, which interacts with the cytoskeleton and intracellular proteins called catenins, must be intact for adhesion to occur. Binding specificity of the cadherins is contained within the first N-terminal domain (Patel and Gumbiner, 1995). X-ray crystallography of the N-terminal domain of murine N-cadherin (NCD1) revealed a two-dimer interface that combines to form a zipper-like structure (Shapiro *et al.*, 1995). It was suggested that this interdigitating zipper-like "supermolecular ribbon" is the basic unit of cadherin adhesive function. To date, cadherins have not been reported to be expressed on endothelial tissue. However, intra-epithelial lymphocytes (IEL) have been found to bind to intestinal epithelial cells *via* the interaction of the $\alpha E\beta 7$ integrin and E-cadherin on epithelial cells (Cepek *et al.*, 1994). Thus, it is possible that cadherins may play a yet-undiscovered role in lymphocyte-endothelial interactions.

(F) Other Adhesion Molecules

Cell adhesion molecules that currently do not fit into the adhesion families discussed above include CD44, VAP-1, and LVAP-2. CD44, which mediates adhesion to hyaluronic acid (HA), is a widely distributed, highly glycosylated integral membrane protein composed of many different isoforms, with molecular weights ranging from

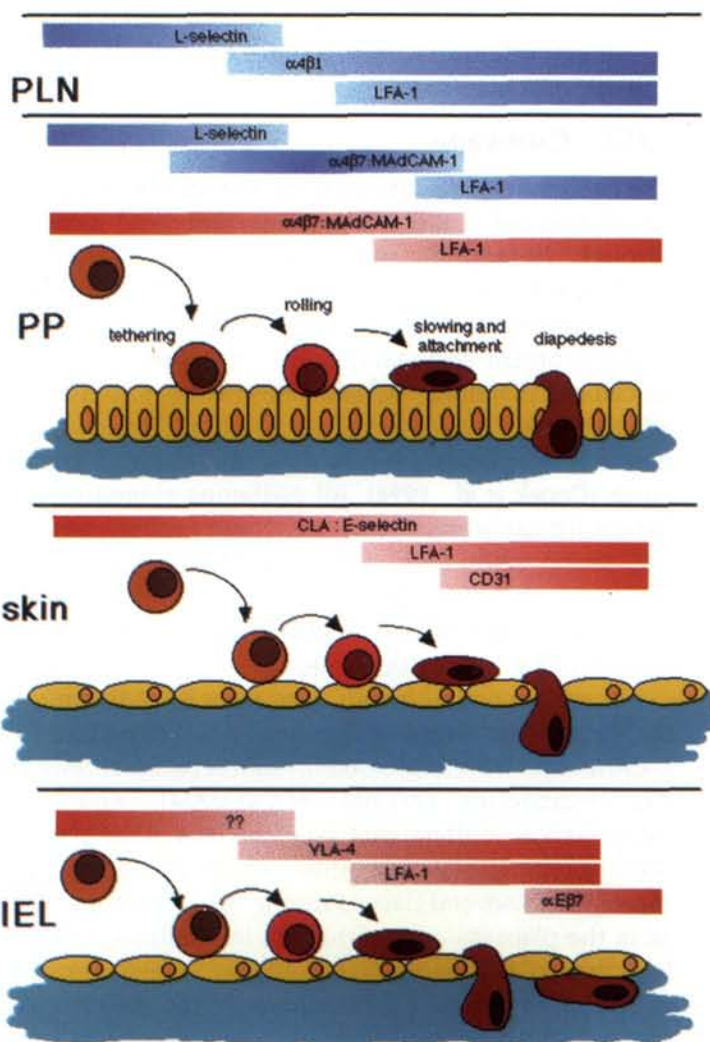

Figure 2. The adhesion cascade. The multistep process of T-cell interaction with endothelium is shown for both naïve (blue) and memory (red) T-cells, including the relevant adhesion molecules. Rolling, attachment, and diapedesis of T-cells through HEV in peripheral lymph nodes (PLN), and Peyer's patches (PP) (top), through endothelium in skin (middle), and of intra-epithelial lymphocytes (IEL) to endothelium in the gut (bottom) are illustrated. The color intensity of the T-cells is intended to illustrate relative states of activation. Light blue, underlying basement membrane/tissue. See text for further details.

85-160 kDa (Lesley *et al.*, 1993). Lymphocytes express the standard 90-kDa form of CD44 at rest and different variable forms with activation (Koopman *et al.*, 1993).

CD44 has been implicated in lymphocyte recirculation, adhesion, and activation. However, the role of the CD44 molecule in these processes is ill-defined. CD44 participates in lymphocyte binding to hyaluronidase-sensitive components on activated ECs. Polyclonal antiserum to CD44 can inhibit lymphocyte binding to HEV. An anti-CD44 mAb, Hermes-3, can block lymphocyte binding to mucosal HEV in a Stamper-Woodruff frozen-section assay, but does not appear to work through the HA pathway, suggesting that this antibody does not block lymphocyte binding to HA. This antibody also shows a potent inhibition of T-cell proliferation in response to phytohemagglutinin, suggesting that Hermes-3 blocks or induces unique signaling events mediated through CD44. Picker *et al.* (1989) showed that leukocyte CD44 and mesenteric LN-derived MAdCAM-1 could interact in solution in a specific, saturable, and reversible manner. However, the Hermes-3 mAb was unable to inhibit lymphocyte binding to purified MAdCAM-1 on Peyer's patch HEV. Lymphocytes that have shed their CD44 are unable to generate the early phase of an anti-hapten delayed-type hypersensitivity (DTH) response, but no effect on lymphocyte recirculation was observed (Camp *et al.*, 1993). Lymphocytes strongly express soluble CD44 (lacking exons 5-14), a 90-kDa, heavily glycosylated secreted protein. Stimulation of T-cells with anti-CD3 mAb, phorbol myristate acetate (PMA), or specific antigen leads to expression of a variety of splice variants, especially those expressing the v6 exon. This exon is also contained in some highly aggressive non-Hodgkin lymphomas. Since activated lymphocytes and lymphomas express v6 variants, they may have similar infiltrative, adhesive, and migratory properties.

Vascular adhesion protein-1 (VAP-1), first identified by use of the mAb 1B2 (made by immunizing mice with human synovium), is a 90-kDa endothelial adhesion protein expressed primarily on HEV and HEV-like venules in PLN, synovium, and tonsils (Jalkanen and Salmi, 1993; Carlos and Harlan, 1994). VAP-1 is not expressed on large-vessel endothelium or human umbilical vein endothelial cells (HUVEC) (McEver, 1994). VAP-1 localization to the lumenal surface of the endothelium, and its granular cytoplasmic staining, suggest that VAP-1 is stored in granules (Jalkanen and Salmi, 1993). VAP-1 is a promising candidate for the proposed synovial vascular addressin. Lymphocyte-vascular adhesion protein-2 (LVAP-2) is a 70-kDa protein expressed on HUVEC, lymphoid and non-lymphoid venules, B-cells, and $CD8^+$ T-cells (McEver, 1994). LVAP-2 is a putative endothelial adhesion molecule for lymphocytes (McEver, 1994). Recent studies have identified LVAP-2 as CD73 (ecto-5'-nucleotidase), a glycosyl phosphatidylinositol-linked molecule that has been previously shown to mediate signals that facilitate T-cell proliferation (Airas *et al.*, 1995).

(III) *The Adhesion Cascade*

The cascade of events that enables lymphocytes to emigrate from the blood into the surrounding tissue is a normal part of T-cell trafficking and is also required in the T-cell response to tissue damage and disease. This process of diapedesis and extravasation is dependent on the expression of specific adhesion and signaling molecules in both the T-cell and the EC. Specific cell-surface receptors, including members of the selectin, integrin, IgSF, and serpentine (7-transmembrane, G protein-coupled) families, are involved in each step of this process. In

addition, successful diapedesis requires the appropriate temporal expression of signaling molecules involved in the activation of both the T-cell and EC.

The adhesion molecules involved in adhesion and diapedesis have been most thoroughly characterized for neutrophil-EC interactions. However, recent data suggest that the same types of interactions, using the same classes of adhesion molecules, are also used by lymphocytes for diapedesis. Previous studies have indicated that there are, in general, four steps in the adhesion cascade: (1) tethering and rolling, (2) activation, (3) stable adherence, and (4) extravasation (Fig. 2). Since recent evidence suggests that different adhesion molecules may be involved in the stabilization of adherence and subsequent extravasation of lymphocytes, these steps will be considered separately. The first step of the process, tethering and rolling, is mediated by interaction of members of the selectin family on both the T-cell and EC and their carbohydrate ligands. This rolling step, which is reversible, permits the lymphocyte to search regions of the vessel wall for the presence of appropriate chemical mediators, or chemokines, that mark sites of inflammation and disease. Interaction of the chemokine with its receptor on the T-cell results, through a G-protein-coupled mechanism, in the activation of integrin molecules on the T-cell (step 2). At the same time, other inflammatory mediators (TNF-α, IL-1β) up-regulate or activate specific cell-adhesion molecules on the EC. Activation of specific integrin molecules on the T-cell results in increased binding of the receptor to its ligand, a member of the IgSF, on the EC. This interaction results in the stable arrest and firm attachment of the T-cell to the EC (step 3). Finally, another integrin:IgSF pair is responsible for the transmigration of the lymphocyte through the vessel wall (step 4). Each of these steps will be described in detail below.

(A) STEP 1: TETHERING AND ROLLING

The initial phase of the adhesion cascade is mediated, in both lymphocytes and neutrophils, to a large extent by the selectins. However, recent evidence suggests that integrin:IgSF interactions may also play a role in this process in some cell populations (see below).

L-selectin is the major T-cell adhesion molecule involved in tethering and rolling. L-selectin binds to peripheral node addressin (PNAd), a mixture of glycoproteins expressed on PLN HEV. These mucin-like ligands, GlyCAM-1 and CD34, contain an epitope recognized by the MECA-79 antibody (Streeter *et al.*, 1988). Sulfation is required for binding of L-selectin to GlyCAM-1 and CD34 (Imai *et al.*, 1993). L-selectin also binds to MAdCAM-1, which is present on mucosal HEV, but not PLN HEV (Berg *et al.*, 1993).

Numerous *in vitro* studies support a role for L-selectin in T-cell tethering and rolling on EC. Lymphocytes, neutrophils, and monocytes will bind to cytokine-activated EC in a Stamper-Woodruff assay, and mAbs to L-selectin are able to reduce this binding (Smith *et al.*, 1991; Spertini *et al.*, 1991b; Brady *et al.*, 1992). Moreover, L-selectin can mediate the attachment of T-lymphocytes under flow to purified MAdCAM-1 coated on a glass plate (Berg *et al.*, 1993). In addition, Lawrence *et al.* (1995) recently showed that PNAd supports lymphocyte and neutrophil tethering and rolling under shear stress. Finally, cells transfected with L-selectin cDNA can induce rolling in the absence of both E- and P-selectin (Ley *et al.*, 1993).

More recent studies have focused on the role of L-selectin in tethering and rolling by means of *in vivo* systems. Mice made deficient in L-selectin expression show a reduction in the number of leukocytes found in PLN, and a reduced number of lymphocytes in lymphatic tissue (Arbones *et al.*, 1994). In these mice, lymphocytes were unable to attach to PLN HEV in *in vitro* binding assays. However, PLN still contained about 30% of the normal number of lymphocytes in this tissue, indicating that some lymphocytes can enter PLN through an L-selectin-independent mechanism. These data suggest that other adhesion molecules may be involved in tethering and rolling. This same study showed that there was a 70% decrease in the number of rolling cells, as determined by intravital microscopy, suggesting that L-selectin is involved in the initial stages of the adhesion cascade.

Although L-selectin is a major player in the initiation of the adhesion cascade in T-cells, there is clear evidence that other adhesion molecules are also involved in this process. Many memory T-cells are L-selectin-negative, and thus, must tether and roll using other adhesion molecules. *In vitro* studies using parallel-plate flow chambers have shown that L-cells transfected with E-selectin will support primary adherence of T-cells (Jones *et al.*, 1994), whereas ICAM-1 transfectants could mediate only stable adherence. When HUVECs were used in the same assay, there was a significant amount of heterogeneity in the adherent behavior of peripheral blood T-cells under flow. This may be the result of the functionally and phenotypically distinct populations of T-cells present in peripheral blood. The authors were unable to identify a mAb that could block primary adherence, thus excluding a role for L-selectin, VCAM-1, $\alpha 4\beta 1$, $\alpha 4\beta 7$, MAdCAM-1, and CD44 in this system. In addition, Lawrence *et al.* (1995) showed that E-selectin can mediate T-cell tethering and rolling under shear flow conditions. Studies with the L-selectin-deficient mice also indicate that other adhesion molecules are involved in primary adherence (Arbones *et al.*, 1994). While there was a significant decrease in the number of cells entering the PLN, this effect was reduced in mesenteric LN, suggesting that expression of MAdCAM-1 in this tissue may mediate primary adherence of $\alpha 4\beta 7^+$

T-cells. The integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$ may be uniquely adapted to play roles in both primary (tethering and rolling) and secondary (stable arrest) adhesion steps.

Indeed, several recent studies clearly show that $\alpha 4$ integrins may play a role in initial tethering and rolling. As mentioned above, Jones *et al.* (1994) showed that while E-selectin- and ICAM-1-transfected L-cells could mediate only primary and secondary T-cell adherence, respectively, VCAM-1 transfectants could mediate both. In another study, Berlin *et al.* (1993) showed that $\alpha 4\beta 7$ could support rapid binding and rolling of cells to recombinant MAdCAM-1 under shear flow *in vitro*, and that this binding was independent of selectins. In addition, activated lymphocytes were shown to bind and roll on venules in the lamina propria of the small intestine, *in situ*, through an $\alpha 4\beta 7$:MAdCAM-1 interaction. Antibodies to $\alpha 4$ or MAdCAM-1 completely blocked these interactions, whereas anti-E-selectin or anti-LFA-1 antibodies had no effect. Recombinant VCAM-1 was also capable of supporting primary adhesion of resting or activated LN cells in a selectin-independent manner. Finally, cells expressing either $\alpha 41$ or $\alpha 4\beta 7$ bound VCAM-1 under flow.

During initial attachment, T-cells flowing through the vessel must be capable of attaching to ligands on the EC wall in a fraction of a second. Slow rolling of the lymphocyte on the cell wall allows the T-cell to inspect the area for the presence of appropriate chemical mediators. Electron microscopy studies have shown that L-selectin is expressed at the tips of microvilli on lymphocytes (Picker *et al.*, 1991). This part of the cell would be the first to come into contact with the cell wall. Concentration of L-selectin in microvilli may increase the avidity of the interaction of L-selectin with its ligand. This localization may also position L-selectin above the glycocalyx and allow for more rapid interaction with ligand. Lawrence and Springer (1991) hypothesized that, to facilitate rolling, there must be a continual series of breaking and formation of interactions between the selectins and their ligands. The prediction that selectins have rapid association and dissociation rate constants has been supported by recent studies (Ushiyama *et al.*, 1993; Alon *et al.*, 1995; Lawrence *et al.*, 1995). These studies showed that lymphocytes attach to and roll on PNAd, associating and dissociating quite easily. However, on average, the T-cells were tethered greater than 95% of the time. Several studies have suggested that L-selectin function is dependent on an affinity change in the molecule (Spertini *et al.*, 1991a), and that this activation requires the L-selectin cytoplasmic domain (Kansas *et al.*, 1993).

While it is clear that expression of L-selectin facilitates lymphocyte trafficking through interaction with its EC ligand, the lack of L-selectin expression on other T-cells may also play a role in overall trafficking. While there was a decrease in the number of lymphocytes in PLN of L-selectin-negative mice, there was a similar increase in the number of cells found in Peyer's patches and spleen (Arbones *et al.*, 1994). These data suggest that because the lymphocytes did not traffic to the PLN HEV, there was a greater probability that cells would traffic to other tissues. It has been suggested that soluble L-selectin, as well as soluble ligands for L-selectin (*i.e.*, soluble GlyCAM-1), may play a role in T-cell trafficking (Brustein *et al.*, 1992; Schleiffenbaum *et al.*, 1992). The potential role of these molecules in T-cell trafficking will be discussed below.

(B) Step 2: Activation

The second phase of the adhesion and transmigration process involves activation of T-cell adhesiveness, primarily through signaling molecules called chemokines. In addition to inducing increased adhesion, these peptide chemoattractants serve to direct the T-cell to the site of tissue destruction or disease. Chemokines are small (70-100 amino acid) peptides expressed by a variety of cell types, including T-cells, monocytes, eosinophils, basophils, mast cells, EC, smooth muscle cells, fibroblasts, keratinocytes, and chondrocytes (Springer, 1994). Chemokines fall into two distinct classes, the α (C-X-C) and β (C-C) chemokines, based on sequence similarity and the amino acid sequence around the first two cysteine residues in the molecule. The α chemokines act primarily on neutrophils; IL-8 is the prototype chemokine of this family. The β chemokines act primarily on monocytes, lymphocytes, and eosinophils, and include monocyte chemoattractant proteins 1, 2, and 3 (MCP-1, 2, 3), macrophage-inhibitory protein-1 α and β (MIP-1α and β), RANTES (regulated on activation normal T expressed and secreted), and eotaxin. In a recent study, MCP-1 was shown to be a major chemoattractant for peripheral blood T-cells (Carr *et al.*, 1994).

Chemokines are found at sites of inflammation and disease and help direct appropriate effector cells to these sites. In response to the chemokine, leukocytes travel in the direction of increasing concentrations of the chemoattractant, being able to detect a 1% concentration gradient across the diameter of the cell. T-cells come into contact with chemokines while rolling on the vessel wall. The precise mechanism by which T-cells come into contact with chemokines is not clear. One possibility is that chemokines are simply released into the blood vessel and T-cells are able to bind as a result of the higher local concentration in that area. Alternatively, there is evidence that chemokines may be presented to T-cells by proteoglycans in the ECM (Gilat *et al.*, 1994), cell-surface proteoglycans, such as CD44 (Tanaka *et al.*, 1993a), or promiscuous chemokine receptors, such as the Duffy blood group antigen (Horuk *et al.*, 1994). Heparin binding sites on the chemokines may provide a site for interaction with proteoglycans. Chemokine presentation may

permit an additional layer of diversity and specificity to develop in the adhesion cascade.

Chemokines bind to cells through specific serpentine (seven-transmembrane), G-coupled protein receptors. Engagement of the receptor by the chemokine leads to a rapid (1-3 seconds) activation of the cell-surface integrin molecules, α4β1, α4β7, and LFA-1. This increased adhesion is accomplished without an increase in the number of adhesion molecules on the cell surface (see below). Coupling of the chemokine receptor to G proteins (usually $G\alpha_i$) is essential. Treatment of cells with pertussis toxin, a specific inhibitor of $G\alpha_i$, leads to an exclusive rolling phenotype (Bargatze and Butcher, 1993), in which rolling is prolonged and there is a complete block of stable arrest in treated cells.

Although chemokines have been proposed to be the major activating stimulus in the adhesion cascade, the ability of receptor-ligand interactions to transduce signals that up-regulate integrin activity must also be considered in the context of the adhesion cascade model. Engagement of CD31 by soluble bivalent mAb has been reported to be sufficient to up-regulate β1 integrin activity on CD31-expressing T-cells (Tanaka *et al.*, 1992). These studies suggest that engagement of CD31 on T-cells, perhaps *via* CD31 or the αvβ3 integrin on endothelium, may play a role in activating integrin receptors expressed on T-cells that are weakly tethered to the endothelial surface. Other candidate triggering receptors on T-cells are selectins and selectin ligands, since recent findings demonstrate that L-selectin engagement or the interaction of neutrophils with E-selectin results in increased functional activity of β2 integrins (Lo *et al.*, 1991; Simon *et al.*, 1995). In addition, the α4 subunit itself has recently been identified as a ligand for α4β7 and α4β1 (Altevogt *et al.*, 1995). This interaction may represent a novel mechanism for autologous integrin activation through homotypic aggregation. The physiologic relevance of this interaction is unknown. Finally, Jones *et al.* (1994) have shown that T-cells will bind and stably adhere (a step thought to require integrin activation) to L-cells expressing VCAM-1. This result suggests a role for α4β1 engagement in integrin activation. These studies propose an intriguing hypothesis that the molecule(s) mediating initial tethering and rolling may also serve as the stimulus that eventually results in stable arrest.

(C) Step 3: Stable arrest

Activation of the T-cell by chemokines leads to the third step in the adhesion cascade, stable arrest or firm attachment to the endothelium. This event is mediated by activated integrins on the surface of the T-cell (α4β1, α4β7, LFA-1) and IgSF adhesion molecules (VCAM-1 and ICAM-1) on the EC. As mentioned above, the increase in adhesiveness is accomplished without an increase in the number of integrin molecules on the surface of the T-cell. This triggering of a high-avidity state is important in the transition of binding from primary (tethering and rolling) to secondary (stable arrest) adhesion (Jones *et al.*, 1994).

Several receptor-ligand interactions may be involved in stable arrest of T-cells to the vessel wall. Only members of the β1, β2, β3, and β7 integrin family are expressed on T-cells, the key players for EC adhesion being α4β1, α4β7, and LFA-1. LFA-1 binds to ICAM-1 and ICAM-2, both of which are expressed on HEV (Marlin and Springer, 1987; Staunton *et al.*, 1989). Inflammatory mediators, such as TNF-α and IL-1, strongly increase ICAM-1 expression on EC (Dustin *et al.*, 1986; Pober *et al.*, 1986). ICAM-2 expression is high on unstimulated EC cells and is not increased by inflammatory mediators (Staunton *et al.*, 1989). VCAM-1, the ligand for α4β1, is expressed on endothelium activated by inflammatory mediators such as TNF, IL-1β, IL-4, and interferon (IFN)-α (Osborn *et al.*, 1989). In addition, VCAM-1 is expressed in other lymphoid areas (LN, tonsils, intestinal lymphoid tissues), macrophages on the splenic red pulp and fetal thymus, hepatic Kupffer cells, and synovial lining layer cells in the synovium of patients with rheumatoid arthritis.

Stable arrest of T-cells to EC is activation-dependent. Using an *in vitro* flow chamber, Lawrence and Springer (1991) showed that resting T-cells bind to PNAd, but bind to ICAM-1 only following activation. Thus, a second signal is required to activate β2 integrin-dependent binding. Jurkat cells rolling on PNAd could be induced to arrest stably on ICAM-1 following stimulation with PMA. Increased adhesiveness to ICAM-1 occurs in a timeframe of seconds. Jones *et al.* (1994), using a parallel-plate flow chamber, showed that L-cell transfectants expressing VCAM-1 could mediate primary and secondary adherence of T-cells under flow. However, L-cells expressing ICAM-1 could mediate only secondary adherence. This difference in primary adherence between α4β1 and LFA-1 was not due to the activation state of these molecules, since there was no difference in primary adhesion of PMA-treated T-cells on L-cells expressing either VCAM-1 or ICAM-1. This difference is likely due to differences in the structures of these molecules or to the physical properties of the interactions. Recent data suggest that both α4β1:VCAM-1 and LFA-1:ICAM-1 interactions may play a role in stable arrest of T-cells to EC (Jones *et al.*, 1994). No reduction in adherence is observed if either pathway is blocked by mAbs, but there is complete arrest if both pathways are blocked. Either pathway seems to be capable of providing a sufficient number of receptors for stable adhesion. It appears that both sets of integrins may contribute to slowing of cells that roll, but not enough to achieve stable arrest. PMA significantly increases the fraction of cells that adhere stably, confirming the need for integrin activation. Taken together, these data suggest that, initially, integrins are in a low-avidity state, and that for those cells that adhere

stably, there is a transition to a high-avidity state involving one or both of the integrins.

Activation of integrins is accomplished through inside-out signaling (reviewed in Hynes, 1992; Mobley *et al.*, 1993). This process is thought to involve conformational changes that lead to a higher affinity of the integrin for ligand. Lollo *et al.* (1993) have recently shown an increase of approximately 200-fold in the affinity of a subpopulation of LFA-1 molecules following activation. Additional evidence also supports the hypothesis of activation-induced conformational changes in integrin receptors. First, mAbs have been described that recognize integrins only in their activated state. Second, binding of divalent cations (Mg^{++}, Ca^{++}, Mn^{++}) leads to increased adhesion and a conformational change, as detected by increased binding to conformation-specific mAbs. Finally, some integrin-specific mAbs have been developed that enhance, as opposed to block, adhesion, again suggesting that they induce a conformational change. In addition to increased affinity, integrin-mediated adhesion can be enhanced by stimuli that act on events occurring after receptor occupancy, such as clustering of integrin receptors. One recent study did, in fact, demonstrate that while both an activating β1-specific mAb and PMA treatment resulted in increased adhesion of lymphoid cell lines to FN, only the activating β1 mAb caused increased affinity of the FN receptor/ligand interaction (Faull *et al.*, 1994).

The intracellular signaling pathways and mechanisms used in integrin activation are not well understood. PMA induction suggests an involvement of protein kinase C. In addition, elevation of intracellular Ca^{++} or cyclic AMP activates LFA-1. Cross-linking of a variety of T-cell surface antigens (CD3/T-cell receptor, CD28, CD2, CD31, CD7, CD44) also leads to activation of integrins (Mobley *et al.*, 1993). Depending on the surface receptors triggered, different signal transduction pathways seem to be involved. The activation of integrins is transient, which allows for de-adhesion, a process that is likely to be as important, and as complex, as adhesion. In addition, T-cell binding of ligand, *i.e.*, α4β1:VCAM-1, can cause outside-in signaling. This process may be important for subsequent steps in the adhesion cascade, as well as for other important processes such as proliferation and differentiation (Shimizu and Shaw, 1991; Pardi *et al.*, 1992).

(D) Step 4: Diapedesis

The mechanisms involved in diapedesis remain poorly characterized. It is clear that integrin:IgSF interactions are involved in leukocyte diapedesis, since mAbs to β2 integrins could completely abrogate stable adherence and diapedesis (Lawrence and Springer, 1991). The identity and the extent of integrin:IgSF involvement in lymphocyte diapedesis are unknown. It is also thought that diapedesis toward a region of tissue damage is unidirectional. Thus, chemokines are likely candidates to direct this process by providing the gradient for lymphocytes to follow. It is possible that the chemokines involved in activation are used for establishing the chemotactic gradient. Alternatively, it is possible that other chemokines, soluble factors, or tethered cytokines are involved in this process.

(E) Summary

The various constellations of adhesion and signaling molecules expressed by the lymphocyte and EC determine the types of cells that will eventually migrate into the tissue (see below). To a large extent, it is the adhesion molecules that are expressed on a cell that determine whether it will emigrate or not. While it is clear that certain cell-adhesion molecule interactions are dominant in the various steps of the adhesion cascade, there is compelling evidence for overlap in their functions (Fig. 2). In addition, it appears that levels of expression are paramount in the involvement of a particular molecule in any given step. As suggested by Butcher (1993), the differences in expression of these molecules may be quantitative rather than qualitative; it is not whether a cell expresses an adhesion molecule, but the level of expression. If a four-step adhesion cascade is assumed, a cell that shows even a two-fold increase in efficiency at each step will be 16 times more likely to extravasate successfully. Expression of different combinations of adhesion molecules on distinct subclasses of lymphocytes, and on EC in diverse locations, as well as the ability to respond to only certain activation stimuli, allow for extraordinary diversity and specificity in the adhesion cascade.

(IV) *Recirculation Patterns of Peripheral T-cell Subsets*

Peripheral T-cells that have not encountered antigen are considered naïve and can be distinguished by several criteria from memory T-cells, which have undergone antigen-specific activation. The latter set of T-cells can be further divided at least temporally, but also in some phenotypic and functional terms, into recently activated effector cells, and true memory cells that persist after antigenic stimulation.

The expression of distinct surface markers such as CD45 provides a means of differentiating these T-cell subsets. Hence, naïve T-cells express the CD45RA isoform, while memory cells are thought to express the CD45RO isoform (Cerottini and MacDonald, 1989). This conclusion is based on numerous observations (reviewed in Mackay, 1994): (1) T-cell stimulation results in the down-regulation of CD45RA mRNA and up-regulation of CD45RO mRNA; (2) CD45RA expression on the cell surface disappears two to three days after T-cell stimulation, while CD45RO surface expression increases

after one to two days; (3) the frequency of CD45RO$^+$ cells is low in cord blood but is greatly increased in adult peripheral blood (Kern *et al.*, 1994); and (4) there is an age-dependent increase in the percentage of CD45RO$^+$ T-cells in peripheral blood. Although generally useful in distinguishing naïve from memory cells, the conversion from a CD45RA^{hi}CD45RO$^-$ to a CD45RA^{lo}CD45RO^{hi} phenotype may not necessarily be unidirectional (Bell and Sparshott, 1990; Fujii *et al.*, 1992), nor may it be applicable to CD8$^+$ T-cells (Mobley *et al.*, 1994). Indeed, unlike CD4$^+$ T-cells, in CD8$^+$ T-cells the expression of CD45RA or CD45RO does not appear to correlate with the respective naïve and memory phenotypes (Adamthwaite and Cooley, 1994).

Characteristic expression patterns for adhesion molecules, such as selectins and integrins, can also be used to determine the activation state of T-cells, albeit with some exceptions that will be addressed below. These expression patterns reflect the important role adhesion molecules play in dictating the distinct routes by which naïve and memory T-cells traffic. Tight control of T-cell recirculation routes *via* specific interactions between T-cell adhesion molecules and their ligands ensures maximal priming of naïve cells and efficient tissue surveillance by memory cells.

Adhesion molecules, such as α4β1, LFA-1, and ICAM-1, are important for binding of T-cells to endothelium and subsequent extravasation. Concordant with naïve to memory T-cell conversion, these adhesion molecules are up-regulated (reviewed in Mobley *et al.*, 1993). Up-regulation of integrin activity occurs in two ways. First, as described above, integrins show an increased affinity/avidity for their respective ligands upon T-cell activation. Second, activated T-cells up-regulate the expression of integrin genes. While in CD4$^+$ cells the expression of β1, β5, LFA-1, CD44, and CD2 is co-regulated with the expression of CD45RO, CD8$^+$ memory T-cells are much more heterogeneous, insofar as a subset of CD45RA$^-$CD45RO$^-$ CD8$^+$ cells expresses high levels of LFA-1, and the CD8$^+$ LFA-1^{hi} population exhibits bimodal β5 expression (Kern *et al.*, 1994). The ability to bind to and migrate through endothelium correlates with high expression levels of LFA-1 and β1 integrins such as α4β1 (Pietschmann *et al.*, 1992; Brezinschek *et al.*, 1995), and is thus closely associated with memory rather than naïve T-cells. Treatment of T-cells with phorbol ester activates these molecules, allowing for less-differentiated cells to migrate through endothelium (Pietschmann *et al.*, 1992).

(A) THE ROLE OF ADHESION MOLECULES IN NAÏVE T-CELL PRIMING

Naïve T-cells encounter antigen in the peri-arteriolar sheath of the spleen and the paracortex of LN, and are trapped there by interdigitating dendritic cells (IDC), which present antigen (reviewed in Steinman, 1991; Sprent, 1994). A limited set of adhesion molecules appears to play a role in naïve T-cell-IDC clustering. Dendritic cells express high levels of LFA-1, ICAM-1, LFA-3, and β1 integrins (reviewed in Steinman, 1991). LFA-1/ICAM-1 interactions may figure prominently in T-cell interactions with IDC and B cells (reviewed in Metlay *et al.*, 1989). Antibodies to LFA-1 inhibit only B-cell and not IDC clustering with T-cells. These data suggest that superior T-cell clustering with IDC compared with B-cells may be due not only to slightly lower levels of LFA-1 and ICAM-1 on B cells (Cassell and Schwartz, 1994), but also perhaps to (an) additional clustering factor(s) on IDC (Metlay *et al.*, 1989).

(B) DISTINCT RECIRCULATION ROUTES

Although both naïve and memory T-cells circulate throughout the body, they utilize distinct routes as a consequence of the differential expression of a number of adhesion molecules (reviewed in Springer, 1994) (see Fig. 2). Naïve cells migrate homogeneously and virtually exclusively to secondary lymphoid tissues such as PLN, Peyer's patches, tonsils, and spleen, but rarely to extra-lymphoid tissues (Picker, 1994). In contrast, memory cells home to both lymphoid and nonlymphoid tissues (Mackay, 1992).

Naïve T-cells circulating in the blood enter LN by extravasation through HEV, which are characterized by a lining of tall endothelial cells arising from cytokine-induced differentiation of flat endothelial cells. These cells leave LN *via* the efferent lymphatic vessels, move into the thoracic duct, and then back into the blood. Memory T-cells reside primarily in tissues, entering through non-HEV postcapillary venules. These T-cells enter LN *via* afferent lymphatic vessels but exit by the same route as naïve T-cells. Emigration to the spleen has been less well characterized, but does not involve HEV (reviewed in Springer, 1994).

(C) PERIPHERAL LYMPH NODE HOMING AND L-SELECTIN

Movement of naïve T-cells through PLN HEV involves tethering *via* selectins, followed by tight integrin-mediated binding. LFA-1/ICAM-1 interactions appear to be important in this latter step, since a mAb specific for LFA-1 can reduce or even abolish T-cell migration into PLN (Camp *et al.*, 1993). P-selectin appears to precede L-selectin in mediating initial contact of T-cells with vascular endothelium, at least in the case of resting CD4$^+$ cells rolling on TNF-β-activated HUVEC (Luscinskas *et al.*, 1995). The relevance of P-selectin in T-cell rolling on HEV in a non-inflammatory milieu is not clear. On the other hand, the importance of L-selectin for rolling has been clearly demonstrated. L-selectin is expressed on the surfaces of all naïve T-cells and thus is instrumental in

directing these cells to PLN. An important exception has been demonstrated in fetal lambs, in which only about half of all naïve thymic emigrants are L-selectin$^+$ (Witherden *et al.*, 1994). Subsequent L-selectin up-regulation by naïve cells appears to be an antigen-independent component of extrathymic maturation. A large number of mature, naïve ovine CD8$^+$ T-cells remain L-selectin$^-$, which correlates with their reduced presence in LN compared with CD4$^+$ cells (Young *et al.*, 1993). The importance of L-selectin in T-cell trafficking *via* HEV has already been discussed earlier in this review.

Upon T-cell activation, L-selectin is selectively down-regulated: In humans, activated T-cells express L-selectin in a tissue-dependent manner (Picker *et al.*, 1993a). T-cells associated with mucosal lymphoid tissue (*e.g.*, tonsils) down-regulate L-selectin, while T-cells associated with PLN remain L-selectin$^+$. Subsequently, the L-selectin phenotype of memory T-cells is bimodal. L-selectin is also rapidly shed from the cell surface following activation. It has been suggested that this may represent a mechanism by which lymphocyte trafficking could be modulated, rapidly leading to accumulation of cells in the appropriate tissue. Loss of L-selectin *via* shedding may also be a prerequisite for subsequent diapedesis. Similarly, Tedder *et al.* (1995) speculate that L-selectin, shed from activated leukocytes, may play a role as a "buffer" to prevent leukocyte attachment to sites of subacute inflammation.

Compared with CD4$^+$ T-cells, L-selectin expression by CD8$^+$ T-cells displays unique features. In mice with primary Sendai virus pneumonia, mediastinal LN cytotoxic T-lymphocytes (CTL) are predominantly L-selectin$^+$ throughout infection and recovery, while CTL in the respiratory tract are largely L-selectin$^-$ (Hou and Doherty, 1993). Memory CTL then revert to an L-selectin$^+$ phenotype (Tripp *et al.*, 1995). There are conflicting data regarding L-selectin expression by CTL during allograft responses. In allografted sheep, alloantigen-specific CD8$^+$ blasts recirculating from the draining LN are L-selectin$^+$ (Verhagen *et al.*, 1995); however, the range of expression is broad among memory cells, implying that, with time, L-selectin is down-regulated, perhaps to divert memory cells away from PLN and preferentially toward the site of the allograft. In contrast, allospecific cytolytic activity in mice is associated with the L-selectin$^-$CD44$^+$, but not the L-selectin$^+$CD44$^+$, subset of effector cells (Mobley and Dailey, 1992). Moreover, L-selectin down-regulation is reversible, because most alloreactive memory cells are L-selectin$^+$CD44$^+$ (Mobley *et al.*, 1994). L-selectin$^-$ cells, taken during the effector phase of the rejection response and placed in culture in the absence of exogenous stimulation, revert to the L-selectin$^+$ phenotype, reflecting the results of Lepault *et al.* (1994). The question, then, is whether L-selectin$^+$CD8$^+$ effector cells become L-selectin$^-$ memory cells (Verhagen *et al.*, 1995), or L-selectin$^-$CD8$^+$ effector cells become L-selectin$^+$ memory cells (Mobley *et al.*, 1994). Differences in animal models and experimental details may at least partially explain this discrepancy.

(D) Tissue-Specific Homing of Memory T-Cells

Unlike naïve T-cells, memory T-cells do not recirculate homogeneously but instead display a propensity to home to a particular tissue or organ, such as skin, lung, or gut (Mackay, 1992; Springer, 1994). Induction of tissue-specific adhesion receptors is apparently a consequence of antigen priming in LN associated with different tissues, so that T-cells are imprinted to return to these tissues later. Local factors present in the microenvironment, rather than antigen directly, mediate adhesion molecule up-regulation. Since many foreign antigens are limited to particular tissues, the ability of T-cells specific for these antigens to home to the relevant tissue makes immune surveillance economical and efficient.

(1) *Skin-homing lymphocytes*

T-cell homing to skin is largely mediated by interactions between CLA on memory T-cells and E-selectin on endothelium (Berg *et al.*, 1991; Shimizu *et al.*, 1991) (Fig. 2). Greater than 90% of T-cells associated with skin express CLA, while CLA$^+$ cells represent less than 10% of peripheral blood T-cells. Generation of the CD45RA^{lo}CD45RO$^+$CLA$^+$L-selectin$^+$ phenotype correlates with conversion of naïve T-cells to effector cells in peripheral lymphoid tissues, being induced to a lesser extent in tonsil and other LN with mixed peripheral mucosal characteristics, and virtually not at all in mucosal LN (Picker *et al.*, 1993b). A current model proposes that T-cells thus activated to express CLA are recruited to cutaneous sites (*via* CLA/E-selectin interactions), where local factors such as TGF-α1 and IL-6 further augment CLA expression (Picker *et al.*, 1993b). Pro-inflammatory cytokines such as IL-1 and TNF-α induce E-selectin (as well as VCAM-1 and ICAM-1) expression on endothelium (reviewed in Bevilacqua, 1993), enhancing T-cell infiltration into inflamed cutaneous tissue. $\alpha 4\beta 1$ and LFA-1, in conjunction with E-selectin, appear to mediate *in vitro* transendothelial migration across activated HUVEC (Babi *et al.*, 1995). E-selectin engagement of CLA may be a prerequisite for integrin activation. Unlike HUVEC, there is little expression of VCAM-1 in skin endothelium, so that *in vivo*, LFA-1 may be more relevant than $\alpha 4\beta 1$.

(2) *Gut-homing lymphocytes*

T-cells expressing $\alpha 4\beta 7$ home to Peyer's patches and intestine as a consequence of binding to MAdCAM-1 (Berlin *et al.*, 1993; Hamann *et al.*, 1994). MAdCAM-1 is expressed on endothelium in mucosal tissues such as Peyer's patches and intestinal lamina propria (reviewed

in Springer, 1994). MAdCAM-1, *via* its mucin-like domain, also mediates initial T-cell tethering by L-selectin (Berg *et al.*, 1993), as described above.

Recent studies have dissected the role of α4β7 in lymphocyte homing to Peyer's patch HEV. While naïve lymphocytes rely primarily on L-selectin, and to a lesser extent α4β7, for initial contact with endothelium, effector/memory T-cells that have up-regulated α4β7 expression (and down-regulated L-selectin) are able to utilize α4β7 for this purpose (Bargatze *et al.*, 1995). In both cases, subsequent α4β7-mediated rolling and sticking precede, and appear to be necessary for, LFA-1-mediated tight binding and diapedesis (Fig. 2). This reliance on α4β7 is probably indirect, *i.e.*, it provides a mechanism for slowing the T-cell in order for it to respond to local vascular signals required for LFA-1 activation. Furthermore, α4 integrins, in general, appear to be able to mediate initial tethering of T-cells, since both α4β1 and α4β7 were shown to mediate rolling on VCAM-1 and MAdCAM-1 (Berlin *et al.*, 1995). The argument that α4β7 is capable of initiating lymphocyte rolling is strengthened by observations that, like L-selectin, this integrin is localized to microvillous projections (Berlin *et al.*, 1995), which are the initial sites of leukocyte-endothelium contact.

A subset of predominantly $CD8^+$ mucosal T-cells, the intra-epithelial lymphocytes (IEL), are localized on the external surface of the basement membrane at the basolateral surfaces of epithelial cells in mucosal tissues, such as gut, as well as nasal, oral, and genito-urinary tracts (Parker *et al.*, 1992). The hallmark IEL integrin is αEβ7, which mediates the adhesion of IEL to epithelial cells (Cepek *et al.*, 1993) by binding to E-cadherin (Cepek *et al.*, 1994; Karecla *et al.*, 1995). Both LFA-1 and αEβ7 appear to be important for IEL adhesion (Fig. 2). However, they are inversely regulated by TGF-α1 produced by epithelial cells, αEβ7 being up-regulated and LFA-1 down-regulated. Cepek *et al.* (1993) propose a migratory route in which IEL first adhere to vascular ECs, migrate across the endothelium, through the lamina propria, and thence to the epithelium. Inverse regulation may be necessary to arrest LFA-1-mediated migration of IEL once they have reached their intra-epithelial destination and anchor them, *via* αEβ7, to the basolateral membrane of epithelial cells. LFA-1 does not appear to play a role in adhesion of IEL to intestinal epithelium, since an anti-LFA-1 mAb did not inhibit this cellular interaction (Cepek *et al.*, 1994). Interestingly, the β2 integrin p150,95 is expressed in mice on approximately half of all IEL in an activation-dependent manner (Huleatt and Lefrançois, 1995), but not on T-cells in lymphoid compartments. However, the specific function of p150,95 on IEL has yet to be elucidated. The mechanism whereby IEL home to the gut is at present unclear. It has been proposed that the adhesion molecule CD31 may play a role in IEL homing, insofar as it is expressed predominantly on $CD8^+$ cells and induces preferential binding to VCAM-1 *via* α4β1 (Tanaka *et al.*, 1992), an interaction which appears to be necessary for T-cell homing to the gut (Issekutz, 1993). Whether CD31 also mediates gut tropism *via* αEβ7 remains to be determined. CD31 has recently been shown to be a ligand for the αvβ3 integrin and, as such, may mediate heterophilic interactions between leukocytes and endothelium (Piali *et al.*, 1995). In contrast, CD31 is expressed by approximately 50% of peripheral blood lymphocytes, which are almost exclusively $CD45RA^+$, and studies indicate that among peripheral blood lymphocytes, CD31 expression appears to be irrelevant to HUVEC transmigration (Bird *et al.*, 1993).

TCR-$\alpha\delta^+$ cells localize primarily to epithelial tissues and comprise a subset of gut lymphocytes. The trafficking of TCR-$\alpha\delta^+$ cells has not been studied as extensively as that of TCR-$\alpha\beta^+$ cells, but evidence indicates that tissue tropism of this T-cell subset may be ontogenetically determined and less reliant on antigen conditioning. For example, TCR-$\alpha\delta^+$ (but not TCR-$\alpha\beta^+$) IEL are found in epithelium from fetal as well as germ-free mice (cited in Cepek *et al.*, 1993). TCR-$\alpha\delta^+$ cells express high levels of L-selectin and home preferentially to LN HEV regardless of their tissue origin (Washington *et al.*, 1994).

In summary, naïve and effector/memory T-cells follow distinct migration routes. This is achieved, on the one hand, by the differential expression of a number of adhesion molecules, including selectins, integrins, IgSF members, and proteoglycans, on naïve, effector, and memory T-cells, and, on the other hand, by the restricted expression patterns of adhesion molecule ligands on endothelium. Thus, adhesion and subsequent transendothelial migration occur in a tissue-specific manner. This is important for the recruitment of naïve cells to secondary lymphoid tissues such as PLN, spleen, Peyers patches, and tonsils, where there are optimal conditions for antigenic stimulation, and for memory cells to be guided to tissues where they are most likely to see those antigens that they have previously encountered, thereby maximizing the efficiency with which T-cells patrol the body.

(V) T-*lymphocyte* Interactions *with* Extracellular Matrix

The ECM is a complex and highly organized mixture of fibrous proteins (such as collagens, laminin, and FN) and glycosaminoglycans (such as HA and chondroitin sulfate) (Mignati and Rifkin, 1993). With the exception of HA, the major glycosyaminoglycans found in the ECM are covalently bound to protein backbones to form proteoglycans. The role of the ECM in providing a structural scaffold for cell growth and development is well-established. For example, the basement membrane, a specialized type of ECM that consists primarily of type IV colla-

gen and laminin, serves as a barrier between epithelial and ECs and the underlying tissue. However, recent studies have clearly revealed the ECM to possess multiple functions in addition to that of a structural framework. For circulating cells, such as lymphocytes, the ECM serves multiple purposes. In this section, we highlight several functions of the ECM relevant to T-cell migration and activation: (1) ECM proteins as specific ligands facilitating T-cell diapedesis; (2) ECM proteins as T-cell chemoattractants; (3) T-cell signal transduction initiated by interaction with ECM components or soluble factors "presented" to T-cells by the ECM; (4) production of ECM proteins by T-cells; and (5) tissue-specific differences in the ECM that may contribute to tissue-specific T-cell migration and/or signal transduction.

(A) THE EXTRACELLULAR MATRIX AND T-LYMPHOCYTE MIGRATION

(1) *Diapedesis*

The final step in the successful exit of lymphocytes out of the bloodstream into lymphoid organs or non-lymphoid tissue sites involves T-cell penetration through the underlying basement membrane and into the surrounding interstitial stroma (Ager, 1994). The precise mechanism by which T-cells carry out this process of diapedesis or transmigration remains poorly defined, although it is now clear that T-cells express several functionally active receptors that can interact with major components of the ECM. Resting peripheral T-cells express the $\alpha4\beta1$ and $\alpha5\beta1$ integrins, which have been shown to mediate activation-dependent T-cell adhesion to FN (Shimizu *et al.*, 1990c). These two integrins bind to distinct sites on the FN molecule, the CS-1 sequence in the type III connecting segment of the major cell adhesion domain (for $\alpha4\beta1$) and the arg-gly-asp (RGD) sequence (for $\alpha5\beta1$). The $\alpha4\beta1$ and $\alpha5\beta1$ integrins have also been reported to mediate activation-dependent binding of human T-cells to thrombospondin (TSP) (Yabkowitz *et al.*, 1993), an adhesive protein that is secreted by activated ECs and activated platelets, and consequently found in high concentrations in the ECM in damaged and inflamed tissues (Mosher, 1990). An additional receptor that mediates the adhesion of resting T-cells to TSP has also been implicated, although the identity of this receptor(s) currently remains unknown. The $\alpha6\beta1$ integrin is also expressed on resting T-cells and mediates T-cell adhesion to laminin (Shimizu *et al.*, 1990c), a major component of the basement membrane. Although resting peripheral T-cells do not bind to collagen in *in vitro* adhesion assays, activation of T-cells *in vitro* results in induction of expression of the $\alpha2\beta1$ integrin (Hemler, 1990) and subsequent $\alpha2\beta1$-mediated binding to collagen (Goldman *et al.*, 1992). CD44 is also expressed at high levels on resting human T-cells (Lesley *et al.*, 1993). The HA-binding activity of CD44 expressed on T-cells appears to be tightly regulated, since resting CD44-positive T-cells do not bind to soluble or immobilized HA (Lesley *et al.*, 1993). Various modes of activating T-cells *in vitro* failed to induce HA binding, although stimulation of T-cells with certain CD44-specific mAbs did result in HA binding (Lesley *et al.*, 1993). Furthermore, transient T-cell binding of HA, following an *in vivo* allogeneic response, has been demonstrated (Lesley *et al.*, 1994), suggesting a physiological relevance for T-cell interactions with HA. ICAM-1, which is expressed at low levels on resting T-cells, has also been reported to be a HA receptor (McCourt *et al.*, 1994). The physiological significance of this finding remains to be established.

Although numerous *in vitro* studies have established that T-cells express multiple functional ECM receptors, the relevance of these cell-ECM interactions during the specific process of diapedesis is not as well established. Both specific antibodies and peptides containing the relevant $\alpha4\beta1$ and $\alpha5\beta1$ binding sequences on FN (CS1 and RGD, respectively) have been shown to inhibit T-cell adhesion to, and migration through, cultured high EC *in vitro* (Ager and Humphries, 1990; Szekanecz *et al.*, 1992; Hourihan *et al.*, 1993). These studies suggest that FN is accessible and available for interaction with T-cells interacting with endothelium in the bloodstream. Consistent with this hypothesis, numerous studies have shown that treatment with FN peptides or integrin-specific antibodies can inhibit T-cell-mediated immune responses *in vivo* (Ferguson *et al.*, 1991; Ferguson and Kupper, 1993; Hershkoviz *et al.*, 1994b; Hines *et al.*, 1994). Although the general conclusion that can be drawn from these studies is that these inhibitory reagents inhibit T-cell migration or movement rather than T-cell activation, the precise mechanism by which these reagents inhibit T-cell trafficking remains difficult to establish in these models.

(2) *Extracellular matrix proteins as chemoattractants*

In addition to serving as specific adhesive substrates, ECM components may also promote T-cell migration by serving as specific chemoattractants. Studies with several T-cell lines and PMA-stimulated T-cells in Boyden chambers have shown both haptotactic and chemotactic migration of these cells on FN, collagen type IV, and laminin (Hauzenberger *et al.*, 1994). These studies also suggested that migration of specific T-cell lines on FN was mediated by either $\alpha4\beta1$ or $\alpha5\beta1$ but not both integrins, even though these cells expressed both of the FN-binding integrins (Hauzenberger *et al.*, 1994). Locomotion of T-cells in a three-dimensional collagen matrix has also been investigated (Friedl *et al.*, 1995). Recent studies in this system have shown that growth of T-cells in this collagen matrix results in the generation of a novel T-cell population characterized by expression of the $\alpha2\beta1$ integrin, which is typically not expressed on

human peripheral T-cells, and low expression of CD44 and L-selectin (Friedl *et al.*, 1995). These results provide additional evidence that T-cell interactions with the ECM are of relevance to differentiation-induced expression of adhesion molecules. The relevance of $\alpha2\beta1$ expression for T-cell movement in this matrix is suggested by the ability of an $\alpha2$-specific mAb to inhibit locomotion.

Recent findings, demonstrating that T-cell adhesion can be regulated by chemokines bound in a biologically active state to proteoglycans (Tanaka *et al.*, 1993b), suggest that the ECM may serve as a reservoir for additional chemoattractants above and beyond the ECM proteins that themselves facilitate T-cell migration. The concept of the ECM as a "cytokine reservoir" has several significant implications for our understanding of the role of the ECM in T-cell migration. First, immobilization of soluble factors in the ECM may be critical for providing a sufficiently high local concentration to allow T-cells to respond to the chemotactic factor. Second, immobilization of such soluble factors in the ECM may result in the generation of chemotactic gradients that may be necessary for facilitating T-cell transmigration through ECs and into the surrounding tissue. Third, this concept illustrates that the biological properties of the ECM are likely to be significantly different in various anatomic locations, since the cytokine milieu differs from tissue to tissue. This may be particularly relevant to our understanding of T-cell migration into sites of inflammation, where the production of cytokines is a biological response of many different cell types involved in inflammation.

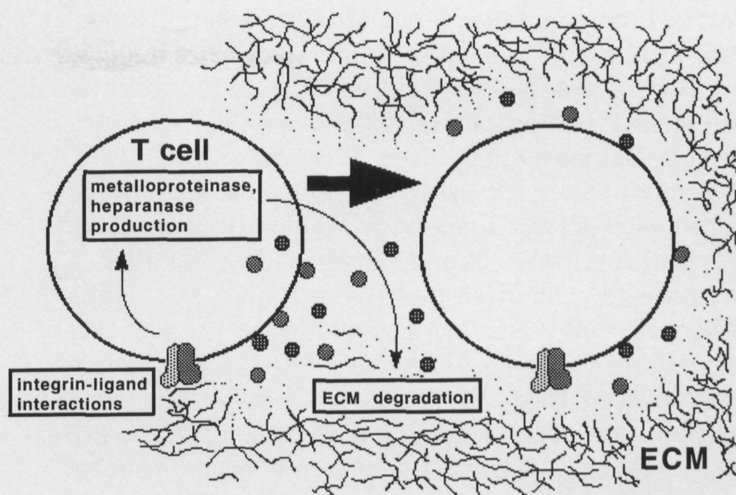

Figure 3. Lymphocyte transmigration and extracellular matrix degradation. Adhesion of T-cells to ECM components such as FN mediates signals that induce the expression of matrix-degrading enzymes such as metalloproteinases and heparanase. The action of these enzymes permits the T-cells to traverse the basement membrane. See text for details.

(3) *Lymphocyte Transmigration and ECM Degradation*

Studies of tumor cells have demonstrated that their production of enzymes capable of degrading ECM components plays a vital role in the cells' ability to metastasize (Mignati and Rifkin, 1993). In addition to tumor cell invasion, enzymatic degradation of the ECM has also been shown to be important in tissue remodeling, during development as well as during wound healing and inflammation, and in angiogenesis (Woessner, 1991). Given that T-cells, during the process of migration, must successfully complete the same types of adhesive interactions as do metastasizing tumor cells, it is not surprising to find that: (1) T-cells produce ECM-degrading enzymes; (2) T-cell production of ECM-degrading enzymes can be regulated by cell-ECM interactions; and (3) ECM-degrading enzymes have been shown to play a role in T-cell migration (Fig. 3).

Several recent studies have documented regulated production, by T-cells, of gelatinase A (72-kD collagenase, matrix metalloproteinase-2), which degrades type IV collagen, and gelatinase B (92-kDa collagenase, matrix metalloproteinase-9), which degrades type V collagen. Leppert *et al.* (1995) reported constitutive expression of gelatinase B by resting T-cells. Stimulation of T-cells with IL-2 resulted in increased gelatinase B expression as well as induced expression of gelatinase A (Leppert *et al.*, 1995). The relevance of gelatinase production for T-cell migration is suggested by the ability of a matrix metalloproteinase inhibitor to block the *in vitro* migration of T-cells across a filter coated with Matrigel, a commonly used commercial reagent derived from a mouse tumor cell line that resembles basement membrane in that it contains predominantly type IV collagen and laminin (Leppert *et al.*, 1995). Inhibition of gelatinase was also shown to block the migration of a mouse T-cell clone through an endothelial monolayer expressing VCAM-1 (Romanic and Madri, 1994). Interestingly, these investigators also demonstrated the presence of gelatinase on the surfaces of these T-cells, and propose that cell-surface association of matrix-degrading enzymes, specifically in the pericellular region, may be necessary for T-cell migration into tissue (Romanic and Madri, 1994).

The expression of matrix metalloproteinases such as gelatinases A and B has been shown to be regulated by integrin receptor-ligand interactions. $\alpha4\beta1$ interaction with VCAM-1 has been shown to induce gelatinase expression in mouse T-cells (Romanic and Madri, 1994). These results are consistent with studies conducted using other cell types demonstrating that integrin engagement can modulate the expression of matrix metalloproteinases (Seftor *et al.*, 1992; Seltzer *et al.*, 1994; Huhtala *et al.*, 1995). Thus, a regulatory network exists whereby cell adhesion generates integrin-mediated signals that serve to induce expression of enzymes involved in degrading the surrounding matrix (Fig. 3). The studies of gelatinase expression in T-cells suggest that this mode of regulation provides an elegant system whereby these

enzymes are produced when matrix degradation is necessary to traverse the basement membrane successfully.

T-cell activation has also been reported to result in the release of heparanase, an enzyme that degrades heparan sulfate proteoglycans (Vlodavsky *et al.*, 1992). Treatment of mice with heparin, which inhibits heparanase activity, was found to inhibit the expression of heparanase by T-cells and to inhibit T-cell migration in response to a DTH reaction (Lider *et al.*, 1990). These results also implicate ECM degradation as critical to successful T-cell migration. Recent studies of heparanase have suggested that conditions in the local tissue environment, specifically pH, can modulate the biological activity of this molecule. Degradation of heparan sulfate proteoglycans by heparanase occurred at acidic pH values ranging from 5.4 to 6.8, with maximal enzymatic activity occurring at pH 6.2-6.8 for heparanase isolated from activated T-cells (Gilat *et al.*, 1995). Activity was minimal at pH values above and below this range. Although not enzymatically active, heparanase was able to bind to ECM at pH 7.2 and could mediate the adhesion of resting T-cells, presumably by T-cell heparan sulfate proteoglycans binding to the ECM-immobilized heparanase. Thus, this molecule can serve as either a matrix-degrading enzyme or an adhesive substrate, depending on the local environmental conditions. It will be important, in the future, to determine if other adhesive factors exhibit similar multidimensional properties.

(B) THE EXTRACELLULAR MATRIX AND LYMPHOCYTE MOVEMENT IN TISSUE

In addition to playing an important structural and regulatory role in T-cell attachment to and transmigration through ECs, the ECM also clearly regulates T-cell movement in tissue. Activation of T-cells, either *via* the antigen-specific TCR or *via* several other cell-surface receptors that have been implicated as being important in antigen-specific T-cell responses, has been shown to result in a rapid and transient increase in β1 integrin-mediated adhesion of T-cells to FN and laminin (Shimizu *et al.*, 1990c; Chan *et al.*, 1991; Mobley *et al.*, 1993). The ability of T-cell activation to increase integrin-mediated T-cell adhesion rapidly to the surrounding ECM has been proposed to be important in the retention and localization of T-cells at the site of antigen encounter (Shimizu *et al.*, 1990a,c). This hypothesis is consistent with studies demonstrating the preferential retention of antigen-specific T-cells in LN draining the site of antigen administration during the first one to two days after introduction of antigen (reviewed in Picker and Butcher, 1992). T-cell activation during an allogeneic response, *in vivo*, has also been shown transiently to activate CD44-mediated binding of T-cells to HA, providing additional evidence that activation-dependent adhesion to the surrounding ECM is likely to play a critical role in the generation and maintenance of a T-cell-specific immune response in tissue (Lesley *et al.*, 1994).

Expression on the cell surfaces of ECM receptors not normally found on the majority of peripheral T-cells may result in the long-term retention of specific T-cell populations in a particular anatomic location. The identification of α1β1-positive T-cells in lung epithelium has led to the suggestion that α1β1 may play a role (perhaps by binding to collagen) in the retention of these cells in the lung (Saltini *et al.*, 1988). Recent studies demonstrating the expression of the αEβ7 integrin on IEL have also led to the hypothesis that the retention of this unique population of T-cells in this site may be due, in whole or in part, to αEβ7 binding to E-cadherin (Cepek *et al.*, 1993, 1994; Parker *et al.*, 1992). This concept of specific retention of T-cell populations by ECM-mediated interactions may also be applicable to our understanding of autoimmune diseases such as arthritis, where the expression of constitutively active integrin receptors due to chronic activation *in vivo* has been proposed to be one mechanism by which autoreactive T-cells are retained in the arthritic joint (Laffón *et al.*, 1991; Rodriguez *et al.*, 1992). Thus, interactions with the ECM may play a role in inhibiting, as well as facilitating, T-cell movement in tissue sites.

(C) THE EXTRACELLULAR MATRIX AND T-CELL ACTIVATION

The process of T-cell activation, in response to an encounter with a foreign antigen, involves the generation of a complex array of intracellular signals mediated by cell-surface receptors that ultimately leads to T-cell proliferation and the generation of an appropriate effector function, such as cytokine secretion or cytotoxicity. While the role of T-cell interactions with other cells, such as antigen-presenting cells, in the process of T-cell activation has been extensively studied and appreciated, it is now clear that the surrounding ECM also plays a role in T-cell responses to foreign antigen. While we have discussed the role that the ECM plays in facilitating T-cell movement into and through tissue, T-cell interactions with the ECM also play a role in T-cell activation by the generation of ECM-induced signaling events that can modulate antigen-specific T-cell responses. Thus, the ECM represents a rich milieu of information that T-cells access to ensure that a specific and appropriate response to a foreign antigen in that site is generated.

(1) ECM-*mediated signal transduction in* T-*cells*

The potential role of the ECM in T-cell signal transduction first came into prominence when purified ECM proteins, most notably FN, were evaluated for their ability to facilitate T-cell proliferation induced by antibody crosslinking of the CD3/T-cell receptor (CD3/TCR) complex. These studies were part of a large interest in

immunology in identifying molecules capable of providing "co-stimulatory" signals, defined as signals that could induce T-cell proliferation in combination with CD3/TCR cross-linking when CD3/TCR cross-linking by itself was insufficient to do so (van Seventer *et al.*, 1991). Initial studies found that under conditions where purified T-cells could not proliferate in response to CD3-specific mAbs immobilized on a plastic surface, co-immobilization of purified FN or laminin with the CD3-specific mAb now resulted in vigorous T-cell proliferation (Matsuyama *et al.*, 1989; Davis *et al.*, 1990; Shimizu *et al.*, 1990b). The co-stimulatory effects of ECM proteins have also been observed with thymocytes (Chang *et al.*, 1995; Ticchioni *et al.*, 1995), consistent with other studies demonstrating a role for the ECM in T-cell development in the thymus (Utsumi *et al.*, 1991; Sawada *et al.*, 1992; Salomon *et al.*, 1994). In aggregate, these studies suggest that intracellular signals generated upon interaction of β1 integrins with either FN or laminin can synergize with the "primary" signal generated by CD3/TCR cross-linking and result in T-cell proliferation.

The co-stimulatory signal provided by ECM proteins in this system was most effective when the CD3-specific mAb and the ECM protein were immobilized on the same plastic surface. This led initially to the hypothesis that the ECM proteins did not transduce a unique intracellular signal but rather that the adhesive properties of the ECM protein enhanced the interaction of the T-cells with the CD3-specific mAb, resulting in a stronger primary signal and T-cell proliferation. This particular objection to the role of ECM in T-cell-mediated signal transduction has now been overshadowed by a large and ever-increasing body of evidence, in multiple cell systems, that ECM receptors can generate intracellular signals upon interaction with ECM proteins. Analysis of integrin-mediated signal transduction has been the most extensive and has recently been reviewed (Clark and Brugge, 1995). We highlight here the most relevant recent studies pertaining specifically to T-cell activation and the role of the ECM in this process.

Direct biochemical evidence that cell-ECM interactions lead to the generation of intracellular signals is now available. In fibroblasts and several other non-lymphoid cell lines, engagement of β1 integrins by either specific mAb or ECM ligands leads to the tyrosine phosphorylation of a 125-kDa protein, which has been identified as the tyrosine kinase focal adhesion kinase (FAK) (reviewed in Richardson and Parsons, 1995; Schaller and Parsons, 1994). FAK localizes to the points of contact (focal adhesions) between these adherent cells and the underlying ECM, and has been shown to interact physically with the cytoplasmic domain of the β1 chain (Schaller and Parsons, 1994). Although T-cells are non-adherent cells and do not form classic focal adhesions when interacting with ECM proteins, β1 integrin-mediated phosphorylation of FAK in a T-cell line has been reported (Nojima *et al.*, 1995). MAb cross-linking of the CD3/TCR has also been reported to result in tyrosine phosphorylation of a novel substrate with homology to FAK that has been named fakB (Kanner *et al.*, 1994). It is currently not known whether fakB is tyrosine-phosphorylated in response to β1 integrin-mediated interactions with the ECM.

Studies of β1 integrin-mediated interactions of T-cells with FN have demonstrated rapid tyrosine phosphorylation of proteins in the molecular weight range of 105-115 kDa (Nojima *et al.*, 1992; Ostergaard and Ma, 1995). The precise identity of these proteins remains unknown, although one report shows that they are not recognized by FAK-specific antibodies (Nojima *et al.*, 1995). These tyrosine kinase substrates may be of particular relevance to our understanding of integrin-mediated signaling in lymphocytes, since β1 integrin engagement has been shown to result in tyrosine phosphorylation of substrates of similar molecular weight in B-cells and NK cells as well as primary T-cells, T-cell lines, and T-cell clones (Freedman *et al.*, 1993; Rabinowich *et al.*, 1995). Proteins of a similar molecular weight have also been reported to be tyrosine-phosphorylated in response to engagement of the αvβ3 vitronectin receptor in αβ T-cell clones (Brando and Shevach, 1995). Clearly, the issue of the functional significance of integrin-mediated tyrosine phosphorylation in T-cell activation will require additional analysis, including: (1) the identification of these novel 105-115-kDa substrates that do not appear to be FAK; (2) the precise role of both FAK and fakB in T-cell activation; and (3) identification of other substrates that may be tyrosine-phosphorylated in response to T-cell interactions with ECM. For example, adhesion of fibroblasts to FN results in tyrosine phosphorylation of the cytoskeletal protein paxillin (Clark and Brugge, 1995), as well as tyrosine phosphorylation and activation of mitogen-activated protein (MAP) kinases (Morino *et al.*, 1995). Engagement of the αvβ3 integrin on mouse T-cells has been shown to result in tyrosine phosphorylation of the CD3 β chain (Sturmhöfel *et al.*, 1995). The relevance of such findings for our understanding of the role of the ECM in T-cell activation awaits further analysis.

Other lines of biochemical and molecular evidence for ECM-mediated signal transduction besides tyrosine phosphorylation are also now available. A unique mAb specific for the β1 integrin chain has been shown to increase levels of intracellular cAMP in T-cells stimulated by CD3-specific or CD2-specific mAbs (Groux *et al.*, 1989). Interaction of T-cells with FN has also been shown to result in induction of the AP-1 transcription factor (Yamada *et al.*, 1991), which notably is involved in induction of IL-2 expression upon T-cell activation. Recent studies have provided additional evidence that cell-ECM interactions can lead to induction of gene expression.

Antibody engagement of the $\alpha4\beta1$ integrin on monocytes induced expression of the tissue factor gene as well as the cytokine TNF-α (Fan *et al.*, 1995). These studies are consistent with findings that interaction of T-cells and monocyte, with ECM, purified FN, or laminin, resulted in TNF-α production by these cell types (Hershkoviz *et al.*, 1993). Interestingly, this study also reported enhanced ECM-induced TNF-α production by T-cells and monocytes if the ECM was first damaged by physical scraping. Interaction of mouse T-cell clones with the ECM *via* the $\alpha v\beta3$ integrin has been shown to be required for constitutive cytokine production by these clones *in vitro* (Moulder *et al.*, 1991; Sturmhöfel *et al.*, 1995). As discussed above, contact of cells with ECM proteins has also been shown to result in induction of expression of matrix-degrading enzymes (Seftor *et al.*, 1992; Seltzer *et al.*, 1994; Huhtala *et al.*, 1995). Co-stimulation of T-cells with a combination of CD3-specific mAbs and purified ICAM-1 or VCAM-1 resulted in the induction of expression of the B7-1 and B7-2 counter-receptor CTLA-4 (Damle *et al.*, 1994), raising the possibility that T-cell co-stimulation by ECM proteins may similarly result in the expression of novel cell-surface molecules. Such results clearly support the hypothesis that ECM proteins and other co-stimulatory molecules may play a vital role in dictating the specificity of T-cell activation and resulting effector functions (van Seventer *et al.*, 1991). Finally, recent studies have also implicated $\beta1$ integrins in the regulation of apoptosis (Koopman *et al.*, 1994; Boudreau *et al.*, 1995).

It should be noted here that it is currently not known if all of the ECM-mediated signaling events that have been reported to occur in non-lymphoid cells also occur upon engagement of the same adhesion receptor expressed on T-cells. As an example, recent studies of $\alpha v\beta3$-dependent interactions of osteoclasts with the bone matrix protein osteopontin have demonstrated that osteopontin can induce the activity of the lipid kinase phosphatidylinositol 3-kinase (PI 3-K) (Hruska *et al.*, 1995), an intracellular enzyme that has been implicated in a wide variety of cellular responses, including membrane ruffling and growth-factor-dependent mitogenesis (Kapeller and Cantley, 1994). These studies also provide evidence for an integrin-associated signaling complex in osteoclasts consisting minimally of PI 3-K, the *c-src* proto-oncogene product, and FAK (Hruska *et al.*, 1995). It remains to be determined whether $\alpha v\beta3$ expressed on T-cells is also associated with such a signaling complex, and whether $\alpha v\beta3$ engagement on T-cells by one of its ECM ligands (such as vitronectin or perhaps even osteopontin), leads to activation of similar intracellular signaling mediators.

Although considerable progress has been made in our understanding of ECM-mediated signal transduction in T-cells, much remains to be elucidated. For example, the precise signaling pathways utilized by integrins and other ECM receptors that result in biologically relevant responses, such as cytokine production, expression of novel cell-surface molecules, and apoptosis, remain poorly defined. The gulf remains large between early signaling events induced by the ECM, such as tyrosine phosphorylation, and the later events, such as induction of gene expression. Potential points of convergence and divergence between the known signaling pathways utilized by the antigen-specific TCR, and the signal transduction pathway(s), induced by T-cell interactions with the ECM, also remain unclear. However, one recent study has demonstrated that constitutive cytokine production mediated by the $\alpha v\beta3$ integrin in a mouse $\alpha\beta$ T-cell clone requires the presence of the ζ-chain of the CD3/TCR complex, suggesting that signaling by this integrin involves a key component of the CD3/TCR in the absence of antigen-specific recognition (Sturmhöfel *et al.*, 1995). These investigators propose that interactions of T-cells with the surrounding ECM lead to integrin-mediated T-cell activation that, in the absence of TCR engagement, can lead to T-cell cytokine production. Such constitutive cytokine production may be relevant to our understanding of the role of T-cells in inflammation. It remains to be determined if this clear point of convergence in integrin-mediated and TCR-mediated signaling in this system is unique to the $\alpha v\beta3$ integrin, or is perhaps a more common feature of ECM-mediated signal transduction in T-cells.

(2) *The extracellular matrix as a cytokine reservoir*

The hypothesis that soluble factors such as chemokines can modulate T-cell adhesion to endothelium, by binding to cell-surface glycosaminoglycans and being "presented" to circulating T-cells, raises the possibility that the ECM, which is rich in proteoglycans, may also serve as a reservoir for biologically active soluble factors. Gilat *et al.* (1994) demonstrated that the chemokines, RANTES and MIP-1α, can be bound by ECM, and that the ECM-bound chemokines can modulate T-cell adhesion to ECM. These findings extend the regulatory effects of proteoglycan-immobilized chemokines to T-cell interactions with ECM, as well as T-cell interactions with endothelium.

Binding and presentation of biologically active factors by the ECM to T-cells have also been extended by findings that TNF-α binds to the N-terminal domain of FN, and that bound TNF increases the adhesion of PMA-stimulated T-cells to FN (Alon *et al.*, 1994; Hershkoviz *et al.*, 1994a). Thus, although TNF by itself is not able to induce integrin-mediated T-cell adhesion to FN, it can enhance adhesion induced by a stimulus that increases $\beta1$ integrin activity, such as PMA. Thus, the ECM may play several vital roles in facilitating the biological effects of TNF-α in inflammatory processes. First, the

ECM can induce TNF-α secretion by T-cells, as well as monocytes (Hershkoviz *et al.*, 1993). Second, the ECM can bind TNF-α once it has been produced (Alon *et al.*, 1994), immobilizing it at the site of the response and perhaps maintaining TNF-α levels at a sufficiently high local concentration for it to be maximally effective. Finally, the ECM-bound TNF-α can enhance the adhesion of activated T-cells to the surrounding ECM (Alon *et al.*, 1994; Hershkoviz *et al.*, 1994a). This may serve two important roles: retaining activated T-cells at the site where the TNF-α is being produced, as well as further promoting additional TNF-α production by enhancing cellular interactions with the ECM. Thus, these studies suggest a regulatory loop, whereby the ECM plays a role not only in initiating cytokine production, but also in maximizing its biological effectiveness and ensuring its continued production (Fig. 4).

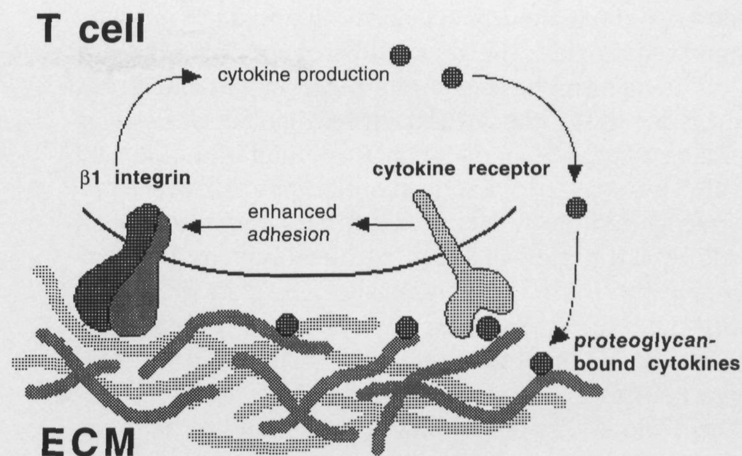

Figure 4. A cytokine-regulatory network involving β1 integrins and the ECM. Cytokines released by T-cells and other cells can bind to proteoglycans in the ECM and subsequently facilitate T-cell migration. In addition, cytokines such as TNF-α can enhance adhesion of activated T-cells to ECM components such as FN. Enhanced T-cell interaction with the ECM, in turn, can lead to increased TNF-α production. See text for details.

(3) *Modulation of lymphocyte activation by* ECM *proteins bound to cell surfaces*

A characteristic of ECM components such as FN, collagen, and laminin is the presence of multiple adhesion-binding sites on a single molecule. This potentially allows a single ECM protein to bind to several different proteins and cells. This, undoubtedly, is necessary for maintaining the structural network that constitutes the ECM. However, several groups have speculated that ECM proteins, particularly FN, might enhance cell-cell adhesion by the recognition of distinct binding domains by different cells, thereby allowing the FN to serve as a cellular "bridge" (Shimizu *et al.*, 1990a; Hershkoviz *et al.*, 1992). This is an appealing concept, since two different cells could potentially bind to one FN molecule, one utilizing the RGD binding site (*via* α5β1) and the other utilizing the CS1 sequence (*via* α4β1). The ECM, thus, might also serve to enhance interactions between T-cells and antigen-presenting cells. Although there is some evidence that FN can be found bound to the cell surfaces of T-cells (Hauzenberger and Sundqvist, 1993), the only evidence that ECM-mediated enhancement of cell-cell adhesion can modulate T-cell activation are studies demonstrating either enhancing or inhibitory effects of soluble FN on various T-cell proliferative responses *in vitro* (Klingemann *et al.*, 1986; Easter *et al.*, 1988; Rybski *et al.*, 1989). The exact mechanism by which FN modulates T-cell activation in these systems has not been extensively analyzed, and thus, the functional relevance, if any, of cell-surface-bound ECM components to T-cell activation remains undefined.

(4) *Activation-dependent production of* ECM *components by lymphocytes*

T-cells have also been shown to be able to produce and secrete FN (Godfrey, 1990; Hershkoviz *et al.*, 1992). Several properties of T-cell-derived FN have been reported, including: (1) agglutination of mononuclear phagocytes (Godfrey, 1990); (2) mediation of neutrophil and monocyte migration through collagen matrices; and (3) enhanced ability to mediate cell adhesion when compared with plasma FN (Hershkoviz *et al.*, 1992). The functional relevance of T-cell production and synthesis of FN for our understanding of T-cell activation remains unclear, although the ability of T-cell activation to induce secretion of FN from T-cells (Hershkoviz *et al.*, 1992) suggests that it may be of some importance.

(D) Functional implications of anatomic heterogeneity in expression of ECM components

In addition to appreciating the relevance of the ECM, in general, to T-cell function, we must also now acknowledge that the ECM can exhibit tissue-specific heterogeneity. This can occur in several ways. First, as discussed in several of the preceding sections, proteoglycans and ECM glycoproteins, such as FN, can bind to soluble factors found in the local environment. Thus, differences in the cytokine milieu, found in one anatomic site *versus* another, can have a profound effect on both T-cell migration and the ensuing activation of those T-cells that have successfully entered that site. Second, there can be unique expression of ECM glycoproteins at certain sites. For example, the ECM protein tenascin has been shown to inhibit certain T-cell-proliferative responses, *in vitro* (Rüegg *et al.*, 1989). Since tenascin can be produced by tumor cells, and can be found to be expressed at sites of tissue injury, these results suggest that local expression of tenascin may, for example, play a role in the response of the immune system to tumor cells. Third, multiple isoforms of both laminin and FN have been identified.

Although the *in vivo* significance of these isoforms is still somewhat unclear, the gain or loss of specific cell adhesion domains in these isoforms suggests that differences in expression of these ECM protein isoforms, between tissues, may have an effect on T-cell migration and activation. One recent study used antibodies that specifically recognized FN molecules containing the alternatively spliced CS1 region of FN to demonstrate that FN containing the α4β1 recognition site (CS1-containing FN) was found to be expressed on EC derived from the synovium of patients suffering from rheumatoid arthritis, but not from normal synovium (Elices *et al.*, 1994). Furthermore, CS1-containing FN was found to be exclusively expressed in arthritic synovium on the lumenal surface of the endothelium, where it was capable of mediating the adhesion of T-cells expressing functionally active α4β1 integrins (Elices *et al.*, 1994). These studies raise the intriguing possibility that α4β1-mediated adhesion of T-cells to CS1-containing FN, found on the lumenal surface of endothelium, may play a pivotal role in the migration of autoreactive T-cells in the synovial fluid, resulting in the generation of this chronic inflammatory condition (Elices *et al.*, 1994). More generally, these results vividly illustrate that elucidation of the anatomic heterogeneity in the ECM, under both normal and diseased conditions, will likely lead to valuable new insights into our understanding of T-cell migration and activation.

(VI) *Clinical Relevance*

Adhesive interactions between T-cells and endothelium are crucial in the host's immune response to infections, inflammation, and other perturbations of the immune system. Unfortunately, these same adhesive interactions can occasionally lead to the development of many human diseases, including vascular thrombosis, diabetes, vasculitis, central nervous system (CNS) inflammation/encephalitis, asthma, tumor metastasis, arthritis, psoriasis, and transplant rejection. Once considered a bystander, the endothelium is now known to be at the center of events leading to an inflammatory lesion. Using recent molecular insights into the adhesion cascade, we are now in a position to begin to understand the events leading to the pathogenesis of many of the above diseases, and to begin to design rational therapeutic strategies to treat them. In this section, we highlight three T-cell-mediated clinical diseases that have been well-studied in the context of lymphocyte-endothelial adhesive interactions: rheumatoid arthritis (RA), psoriasis, and experimental autoimmune encephalomyelitis (EAE).

(A) Rheumatoid Arthritis

Although all humans have autoreactive B- and T-cells, normal regulatory mechanisms (genetic factors, pathogens, etc.) prevent these cells from reacting to self-antigens. It is only when these regulatory mechanisms fail that autoimmune disease occurs (Steinman, 1995). One of the most common autoimmune diseases is RA, a systemic autoimmune disease characterized by a chronic, symmetric, and erosive synovitis of peripheral joints (Wilder, 1993). Although the etiology of RA is unknown, basic research has provided a substantial understanding of the molecular and cellular aspects of pathogenesis. Rheumatoid synovitis involves many mediators and cell types, including the synovium, neutrophils, monocytes, macrophages, cytokines (IL-1, TNF, and IFN-β), ECs, and lymphocytes (Wilder, 1993). The rheumatoid synovial immunoreactive lesion is comprised of a mononuclear cell infiltrate (antigen-presenting cells) in close contact with T lymphocytes (Oppenheimer-Marks and Lipsky, 1995). The phenotype of these synovial tissue lymphocytes is consistent with memory $CD4^+$ cells ($CD45RO^+$, $CD45RA^-$, $CD29^{bright}$, LFA-1^{bright}, $CD44^{bright}$, LFA-3^{bright}, ICAM-1^+, L-$selectin^-$) (Oppenheimer-Marks and Lipsky, 1995). These immunoreactive units are thought to be responsible for the initiation and "perpetuation" of RA.

Rheumatoid synovial endothelium expresses many cell-adhesion molecules, including ICAM-1, VCAM-1, P-selectin, and E-selectin (Oppenheimer-Marks and Lipsky, 1995). There has been a correlation in RA disease activity with soluble VCAM-1, but not ICAM-1, levels, despite the fact that ICAM-1 is the most intensely and broadly expressed adhesion molecule on RA endothelium (Pitzalis *et al.*, 1994; Oppenheimer-Marks and Lipsky, 1995). Therapeutic trials in both animal models and humans have attempted to block the initiation or the continuation of the inflammatory synovial units by abrogating cellular adhesion with blocking peptides or antibodies (Oppenheimer-Marks and Lipsky, 1995). A recent open-label dose-escalation trial of an ICAM-1-specific mAb demonstrated significant long-term clinical improvement in patients with severe refractory RA receiving a five-day treatment (Kavanaugh *et al.*, 1994). Further studies using ICAM-1-specific mAb in RA patients are currently under way.

(B) Psoriasis

Psoriasis is a common idiopathic chronic inflammatory dermatologic disease that affects 1-2% of people and is characterized by erythematous, sharply demarcated papules, and rounded plaques with silvery scales. Important etiologic factors include genetic predisposition, infections (*i.e.*, streptococcal), trauma, and other environmental factors. Immunohistochemical analysis of psoriatic lesions (plaques) reveals epidermal hyperproliferation, changes in the dermal microvasculature (including dilatation and tortuosity of the superficial dermal plexus, angiogenesis, and HEV formation), and an increased number of T-cells (reviewed in Griffiths, 1994; Lowe *et al.*, 1995). Initially misidentified as a nonim-

munologically mediated disease, secondary to the marked epidermal hyperproliferation, psoriasis is now known to be caused by abnormal microvasculature lymphocyte:EC interactions.

In psoriatic lesions, the ECs in the papillary microvessels express increased levels of E-selectin, ICAM-1, and VCAM-1, compared with normal and non-involved psoriatic skin (Griffiths, 1994; Wakita and Takigawa, 1994). Wakita and Takigawa (1994) analyzed psoriatic lesions at different phases of disease [initial (I), active (II), and resolving (III)], and found that E-selectin, ICAM-1, and VCAM-1 were most highly expressed in active phase II lesions. A recent study by Bonifati *et al.* (1995) demonstrated that soluble ICAM-1 levels in serum correlated with disease severity scores (psoriasis area and severity index) in psoriasis patients. The increase in ICAM-1, VCAM-1, and E-selectin levels correlates with the influx of CD4+ T-cells, suggesting that these molecules are important in the trafficking of T-cells into psoriatic lesions. Interestingly, as psoriatic lesions begin to involute and resolve, the CD8:CD4 T-cell ratio increases significantly (Wakita and Takigawa, 1994). The importance of this finding remains unknown.

Analysis of T-cells in psoriatic lesions has led to many interesting findings. Active psoriatic lesions contain an increase in $CD4^{+}CD45RO^{+}$ T-cells bearing activation markers, including HLA-DR and IL-2 receptor (CD25) (Griffiths, 1994). The influx of activated memory T-cells, preceding the characteristic hyperproliferation of the epidermal cells (Wakita and Takigawa, 1994), suggests that T-cells help initiate the inflammatory response. This T-cell-mediated inflammation is thought to result in the release of several cytokines and growth factors presumed to play a role in the pathogenesis of psoriatic plaques, including IFN-γ, TNF-α, and two keratinocyte proliferative factors, IL-8 and transforming growth factor (Griffiths, 1994). T-cells that home to the skin are known to express the E selectin ligand CLA (see above) preferentially. It is likely that the T-cells found in active psoriatic lesions express CLA (Griffiths, 1994). Therefore, the CLA/E-selectin interaction is a prime target for immunopharmocologic therapy in psoriasis.

(C) Experimental Autoimmune Encephalomyelitis

A third example of an autoimmmune disease involving lymphocyte-endothelial adhesive interactions is EAE, an experimental animal model that mimics multiple sclerosis (MS) (reviewed in Yednock *et al.*, 1992). MS is a relapsing-remitting clinical disease that affects myelin-containing white-matter tracts within the CNS and often presents with optic neuritis or motor and sensory impairments (Francis *et al.*, 1991). In both MS and EAE, lymphocytes and monocytes cross the blood-brain barrier, penetrating the brain parenchyma and damaging myelin (Francis *et al.*, 1991). EAE can be induced in animals either by priming with CNS proteins, such as myelin basic protein, or by adoptive transfer of CNS antigen-specific, activated T-cell clones. In EAE, the $CD4^{+}$ memory T-lymphocytes gain access into the brain parenchyma by $\alpha4\beta1$-mediated binding to VCAM-1 expressed on brain vessel endothelium (Yednock *et al.*, 1992). In a rat model of EAE, the delayed (up to two days) administration of anti-$\alpha4$ mAb prevented, or greatly attenuated, disease progression (Yednock *et al.*, 1992). The investigators speculated that the antibody treatment blocked the entry of host monocytes and lymphocytes that normally would have infiltrated the brain in response to the inflammation induced by the T-cell clone. The fact that the antibody could be given after the introduction of the disease-initiating T-cell clone provides hope that similar treatments might work in MS patients, even after the onset of symptoms.

(D) Effects of Known Therapeutic Agents on Adhesion Molecules and New Therapies

The elucidation of adhesive cell-endothelial interactions in RA, psoriasis, and EAE has led to a better understanding of the therapeutic actions of many of the current medications used in immune-mediated diseases, and to the promise of new anti-adhesive therapies. We now know that many of the current anti-inflammatory and immunosuppressive medications, chosen empirically for their clinical efficacy, work by modulating lymphocyte-EC interactions. Corticosteroids, long a mainstay of treatment for RA, are now known to diminish the capacity of endothelium to up-regulate ICAM-1 and E-selectin expression after endotoxin or IL-1 treatment (Cronstein and Weissman, 1993). Colchicine, a phenanthrene derivative, has recently been found to diminish leukocyte rolling in the microvasculature, presumably due to a marked decrease in the expression of L-selectin on leukocytes and ICAM-1 on endothelium (Cronstein and Weissman, 1993). Last, methotrexate (an anti-metabolite) most likely induces an increase in the amount of adenosine, known to decrease $\beta2$ integrin-mediated adhesion, released from injured cells (Cronstein and Weissman, 1993; Oppenheimer-Marks and Lipsky, 1995). Although vital to our current treatment armamentarium, the fact that these medications are unable to cure most immune-mediated diseases has fueled the search for a new class of anti-adhesion pharmaceutics.

Potential therapies currently being tested, *in vitro* and *in vivo*, include: mAbs, soluble receptors, peptide sequences able to block adhesion receptors, adhesion-recognition carbohydrates, and antisense oligonucleotides (Makgoba *et al.*, 1992; Oppenheimer-Marks and Lipsky, 1995). Ultimately, ideal anti-adhesion therapies will have to demonstrate: (1) significant clinical benefit; (2) an effective and feasible dosing route; (3) sufficient

specificity to allow other critical adhesion processes to continue; and (4) a low side-effect profile (Makgoba *et al.*, 1992). Given the multitude of different immune-mediated diseases (acute and chronic) and the many different classes of adhesion molecules involved, it can be expected that several different therapeutic de-adhesive agents and combinations will need to be utilized.

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