

## IMMUNE SURVEILLANCE OF THE ORAL CAVITY AND LYMPHOCYTE MIGRATION: RELEVANCE FOR ALCOHOL ABUSERS

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### ABSTRACT

*The health of the oral cavity is threatened by a variety of microorganisms. Impaired immune surveillance of the oral environment contributes to the development of infectious processes and tumors of the mouth. Elucidation of the physiological mechanisms that determine and control oral immune surveillance is crucial to an understanding of these oral diseases. The ability of lymphocytes to migrate is critical for successful immune surveillance. The cardinal facets of lymphocyte migration are reviewed here in the context of the oral cavity. One mechanism by which alcohol acts as an important cofactor in the onset and development of oral diseases is hypothesized to be through impaired lymphocyte migration to and from peri-oral lymphoid tissue.*

### IMMUNE SURVEILLANCE OF THE ORAL CAVITY

Impaired immune surveillance of the oral environment contributes to the development of cancers of the mouth. The health of the oral cavity is also threatened by a variety of microorganisms (1). Most of these become pathogenic when the host's immune system is altered. This explains the predominance of aggressive oral diseases of fungal, viral and bacterial origins and of oral tumors in an overwhelming number of patients afflicted

with the acquired immunodeficiency syndrome (2,3). The elucidation of the physiological mechanisms that regulate immune surveillance of the oral cavity is crucial to understand a variety of oral diseases, and to develop effective modes of palliative treatment interventions (1).

Immune surveillance is the outcome of concerted events and processes brought about by lymphoid, myeloid and other immune cells, the factors they produce in response to antigenic stimuli, and neural and endocrine modulation of these immune responses. The purpose of this work is not to provide an extensive review of all immune-associated processes and events that occur in the oral cavity. Rather, we focus upon relatively new developments in cellular immunity the importance of which has now been fully appreciated among immunologists: the role of lymphocyte migration in immune surveillance. The proposed hypothesis is that one mechanism by which alcohol acts as a cofactor in the onset and development of oral diseases is by altering lymphocyte migration to and from peri-oral lymphoid tissue.

Lymphocytes are one of the several combatants (albeit some investigators may wish to consider lymphocytes to be the "generals of the immune army") involved in defending the host against fungal, viral and bacterial pathogens, and against carcinogenesis. The oral cavity is one of several sites (albeit some may defend that it represents the

initial and principal port of entry of microbes, such as fungi and viruses) that allow pathogenic invasion. Accordingly, at the onset of this discussion, certain salient features of the immunological system as it relates specifically to immune surveillance mechanisms of the oral cavity are outlined.

In broad terms, the immune system can be viewed as multiple partially overlapping compartments, including the integumentary, the mucosal and the systemic immune compartments. Mucosal immunologists further sub-compartmentalize mucosal immunity in terms of the specific anatomical location, such as for instance the gut-associated, bronchus-associated, middle-ear-associated and oral cavity-associated lymphoid tissues (4). Each immune compartment has several properties and characteristics that distinguish it from the others (e.g., prevalence of certain immune processes, immune cell populations and immune factors vs. their respective paucity in others). This is not to say that immune compartments do not overlap; indeed, they share many features and properties, and several populations of immune cells migrate and recirculate from one compartment to another. One important aspect of immune development and maturation is the ability to confer to challenged immune cells the property and ability to migrate preferentially and specifically to the site where the original challenge took place. This increases the likelihood for prompt immune recall responses, should the organism be again exposed to that antigenic challenge (5-7). Immunological defense processes responsible for the immune surveillance of the oral cavity primarily belong to the mucosal immune compartment, but the systemic immune system also contributes significantly in this context.

The oral mucosa is the first line of defense of the oral cavity against foreign pathogens. When intact, it effectively prevents penetration by microorganisms. Except in the mucosa of the lips, cheeks, buccal floor and soft aspect of the palatal

roof where keratin is essentially absent, keratin plays an important role in preserving the structural integrity of the oral mucosa. Keratinized and non-keratinized oral epithelium is underlaid by the granular layer and the basement membrane of the epithelium. As is discussed below, leukocytes are found in this epithelium, interspersed within the lamina propria of the buccal cavity.

The principal mucus found in the oral cavity is saliva, a fluid composed of secretions produced by the parotid, submandibular, sublingual and other lesser salivary glands. Saliva is a complex fluid that is important for the maintenance of oral hygiene. It provides essential lubrication of the anatomical structures within the oral cavity during speaking, chewing and swallowing, and is the prime diluent of the pathogens that invade the oral cavity. Their suspension in saliva permits their quick elimination from that site either externally (spitting) or internally (swallowing). The rather inhospitable environment of the gut quickly destroys most of them. A third non-specific immunosurveillance function of saliva is its role as a diluent of factors that participate in the neutralization and destruction of these pathogens within the oral cavity.

Saliva contains lactoferrin, a bacteriostatic protein that sequesters iron molecules otherwise available to pathogens for their replicative cycle (particularly, enzymes responsible for DNA replication and RNA transcription). Saliva also contains lysozyme, the muramidase enzyme that catabolizes the mucopeptide cell wall of bacteria by breaking the bond between N-acetylglucosamine and N-acetyl muramic acid. Lysozyme modulates the response of cellular immune components, such as lymphocyte mediated proliferation and interleukin-2 production. In addition, saliva contains certain complement components, which suggests that complement-mediated immune surveillance occurs within the oral cavity, although this argument still remains a subject of controversy. Secretory immunoglobulin A (IgA) is found in

abundance in saliva. Salivary IgA is produced by lymphoblasts that inhabit the salivary glands. Salivary IgA concentration (on the average 0.2 mg/cc) is about ten times higher than that of salivary IgG and one hundred times that of salivary IgM. Salivary IgA is dimeric. In serum, by contrast (blood constituting an aspect of the systemic immune compartment), IgA is found in the monomeric form at about 2 mg/cc, that is 20% the concentration of serum IgG. Fluid in the gingival crevices, which by most account is an exudate from the gingival and periodontal microvasculature, is also 3-5 times richer in IgG than IgA (about 1 mg/cc). The gingival fluid is also rich in complement components and bacteria-lysing lysosomal enzymes (1,2,8).

The presence of immunoblasts within the salivary glands suggest that these glands could serve as the sites of immunogenic reactivity leading to the maturation of IgA-secreting plasma B cells. This should be consequential to the invasion of the salivary gland duct and parenchyma by pathogens, their processing by residing antigen-presenting cells, their presentation to residing T cells, and sustained T cell-mediated responses leading to the maturation of IgA-secreting B lymphoblasts. There is today little evidence to support the existence of immune events of this magnitude within the salivary glands. Rather, the argument appears more convincing that the presence of plasma cells capable of secreting IgA in the salivary glands may be consequential to the recirculation of pre-sensitized B cells into these sites by means of the integrin-adhesion molecule interactions described below (i.e., salivary gland → saliva intestinal lamina propria → Peyer's patches → mesenteric lymph nodes → thoracic duct → blood → salivary gland microvasculature → salivary gland parenchyma).

Indeed, immune surveillance of the oral cavity is brought about primarily by immune cell populations that transit and recirculate to this anatomical site. Principal but not

exclusive representatives are lymphoid and myeloid descendants, as well as cell populations endowed with natural killer activity (9-11). The focus of this paper is upon the migratory properties of B and T lymphocytes within the oral cavity. Certain properties of the circulation of T cell subsets, identified by the expression of their surface antigen, CD4 (cluster of differentiation # 4) or CD8, to and from the peri-oral lymphoid tissue are described below.

### *PERI-ORAL LYMPHOID TISSUE*

The oral cavity is rich in lymphoid tissue, which insures effective immune surveillance in this anatomical region. The distribution of lymphatic tissue within and around the oral cavity reveals the intricate nature of the events that are responsible for the drainage of the mouth. Intra-oral and extra-oral lymphoid tissue can be identified histologically. Intra-oral lymphoid tissue consists of the submucosal, gingival, salivary and tonsillar lymphoid tissues. A fine network of lymphatic capillaries commences superficially within the oral mucosa and dental pulp, joins deeper larger lymphatic capillaries to form lymphatic vessels that drain afferent lymph to the extra-oral chain of peripheral lymph nodes. Depending upon the anatomical site, this drainage occurs to the extra-oral submental, submandibular, upper superficial and deep cervical, and retropharyngeal lymph nodes.

Extra-oral nodes, which show histological features that are similar to those of peripheral lymph nodes: that is, encapsulated structures that consist of a cortical, paracortical and medullary area, belong to the systemic immune compartment (12). By contrast, intra-oral lymphoid tissue shares histological features primarily with the mucosal immune compartment. The submucosal lymphoid tissue of the oral cavity appears histologically as a diffuse layer of scattered lymphoid cells that resembles the structure and organization of lymphoid tissue within other mucosal

tissues. The gingival lymphoid tissue, particularly evident in association with plaque accumulation or gingivitis, consists primarily of clusters of B and T cells, with the former usually predominating, and activated antigen-presenting macrophages and neutrophils. The salivary gland lymphoid tissue presents, as mentioned above, predominantly IgA-secreting B lymphoblasts scattered between acini and in small clusters adjacent to the salivary ducts.

Tonsillar lymphoid tissue is distinct from intra-oral lymphoid tissue in that it consists of three paired lymphoid aggregates that share several features with the mucosal and with the systemic lymphoid tissue. Tonsils are located as a lymphoid cluster (Waldeyer ring) at the border of the oro- and nasopharynx, and thus act as important gate-keepers of the oral cavity (10,13). The palatine tonsils are located superiorly, between the glosso- and pharyngo-palatine arches. The lingual tonsils reside on each side of the tongue, posterior to the circumvallate papillae. The pharyngeal tonsils (adenoids) are situated inferior to the nasopharyngeal mucosa in the nasopharynx.

Tonsillar lymphoid tissue is not encapsulated and does not have afferent lymphatics, and, in that respect, differs from peripheral lymph nodes. It is covered by a layer of squamous epithelium that invaginates into ten-to-twenty fissures, called crypts. Pathogens and foreign material become trapped within the cryptic fissures. The cryptic epithelium is endowed with increased permeability that allows the transfer of these materials within the lymphoid compartment. Within the peri-follicular parenchyma of the tonsil, pathogens are promptly phagocytosed and processed by residing macrophages, dendritic cells and other antigen-presenting cells. Histological examination of the tonsils reveals the presence of a peripheral zone and a central core. The latter is composed of a lymphoid follicle and a germinal center, both rich in B cells and memory T cells. In that respect, tonsils share with peripheral extra-oral lymph nodes the property of having all

the necessary constituents to mount effective primary and secondary immune responses (10,12,14-16).

Detailed histological examination reveals that both peripheral extra-oral lymph nodes and tonsils are characterized by a first, or cortical, zone that is rich in B and memory T cells. In the node, this zone runs parallel and adjacent to the inner aspect of the capsule and receives migrating lymphocytes by means of afferent lymphatics. These structures are absent in the tonsils, as previously indicated, and to some extent replaced by transfer across the cryptic endothelium. Tonsillar and nodal lymphoid tissue are characterized by the paracortical zone, typically encapsulated by the first in the lymph nodes and by the cryptic epithelium in the tonsils. This zone is rich in naive T cells, which penetrate the parenchyma by means of interaction of membrane receptors with ligands on the high endothelium of specialized venules (see below). The innermost medullary zone is rich in B and memory T cells, which exit the lymph tissue by means of efferent lymphatics. The paracortical area of both nodal and tonsillar tissue houses lymphoid nodules and consists of B cell follicles and adjacent T cell-rich areas. Macrophages and dendritic cells also reside within the follicles. Internodular spaces serve as lymphocyte transit areas. Following antigenic stimulation, T cell-dependent events in the paracortical areas lead to the maturation and proliferation of B cells, and to the formation of germinal centers that house B memory cells. Follicular dendritic cells play a central role in the generation of humoral B cell-mediated immune responses. The latter cell population intimately interacts with B lymphocytes by means of specific membrane bound receptor-ligand interactions that involve integrins and adhesion molecules (see below). These interactions both induce B cell maturation and protect B cells from apoptosis. T cell-independent events lead to the formation of a mantle, or corona, that produces B memory cells against T cell-independent antigens.

Reticular dendritic cells and macrophages are found in large numbers within the germinal centers and the mantle of peripheral extra-oral nodal as well as tonsillar lymphoid tissue (10,11,12,14-17).

Histochemical analyses further demonstrate that tonsillar lymphoid tissues share the property with other lymphoid tissues of rich cholinergic, catecholaminergic and neuropeptidergic innervation (e.g., substance P, neuropeptide Y, vasointestinal peptide). Immunocompetent cells in both preparations can be seen to be in close contact with nerve terminals, suggesting the direct involvement of the nervous system in modulating immune responses at these sites (18-20). Neuroanatomical distribution suggests that neuropeptide Y-immunoreactive fibers, and associated tyrosine hydroxylase staining of sympathetic neurons in the peri-oral lymphoid tissue, originate from the superior cervical ganglion. Vasointestinal peptide-immunoreactive fibers are parasympathetics from the sphenopalatine ganglion that probably relay information from the central nervous system to the tonsillar and extra-oral nodal environment. Substance P fibers belong to a group of sensory fibers, that originate from the maxillary branch of the trigeminal nerve or the dorsal root of the cervical ganglion. These fibers most likely transmit information to the central nervous system about ongoing inflammatory or immune processes within the oral cavity. Substance P innervation abounds in peri-oral mucosa, submucosa and secretory glands. Because of the close association of these nerve fibers to acini and secretory ducts, a significant role for substance P in oral and peri-oral mucus secretion has been proposed in humans and in experimental animals (19,21,22).

The ontogeny of peri-oral lymphoid tissue is similar to that of other lymphoid tissues in that it originates from the hemato-blastemic mesoderm at about the 9th week of fetal development. In the case of tonsillar tissue, endodermal epithelium derived from

the second pharyngeal pouch then begins to form the cryptic fissures. In the instance of extra-oral peripheral lymph nodes, jugular sacs, formed from the endothelium originally part of the vascular plexus of mesodermal origin, lead to the development of the nodal capsule, internodal lymphatics and intra-nodal venules. Tonsils involute with aging, albeit much later than thymic tissue (23,24).

#### *T LYMPHOCYTE RECIRCULATION AND IMMUNE SURVEILLANCE*

The circulation and migration of lymphocytes is a rapid, selective and efficient process mediated, in part, at the molecular level. The interaction between certain lymphocyte membrane receptors and their respective endothelial ligands regulate T and B cell migration and homing patterns. At least three fundamental regulation events direct the traffic of leukocytes to and from lymphoid tissue with multiple combinational diversity (5,6,25-27).

Cell taxis refers to the process of cell migration as a function of the concentration gradient of a given attractant. Chemotaxis refers to tactic phenomena that involve chemokines, substances that diffuse from their anatomical site of production and release. The family of chemokines is vast and includes soluble immune and growth factors, hormones, neuropeptides and other biochemical products (e.g., prostaglandins). The membrane of lymphocytes is endowed with specific receptors for many of these chemokines. The chemotactic response is a function of the chemokine concentration, the number and affinity of receptors on the responding cell's membrane, and the modulatory effects these receptors have upon transmembrane signaling events associated with cell activation. Haptotaxis, by contrast, refers to the migration of cells as a function of substratum adhesiveness. This process is mediated by a large family of endothelial ligands and their membrane receptors expressed by the migrating cell. Lymphocyte

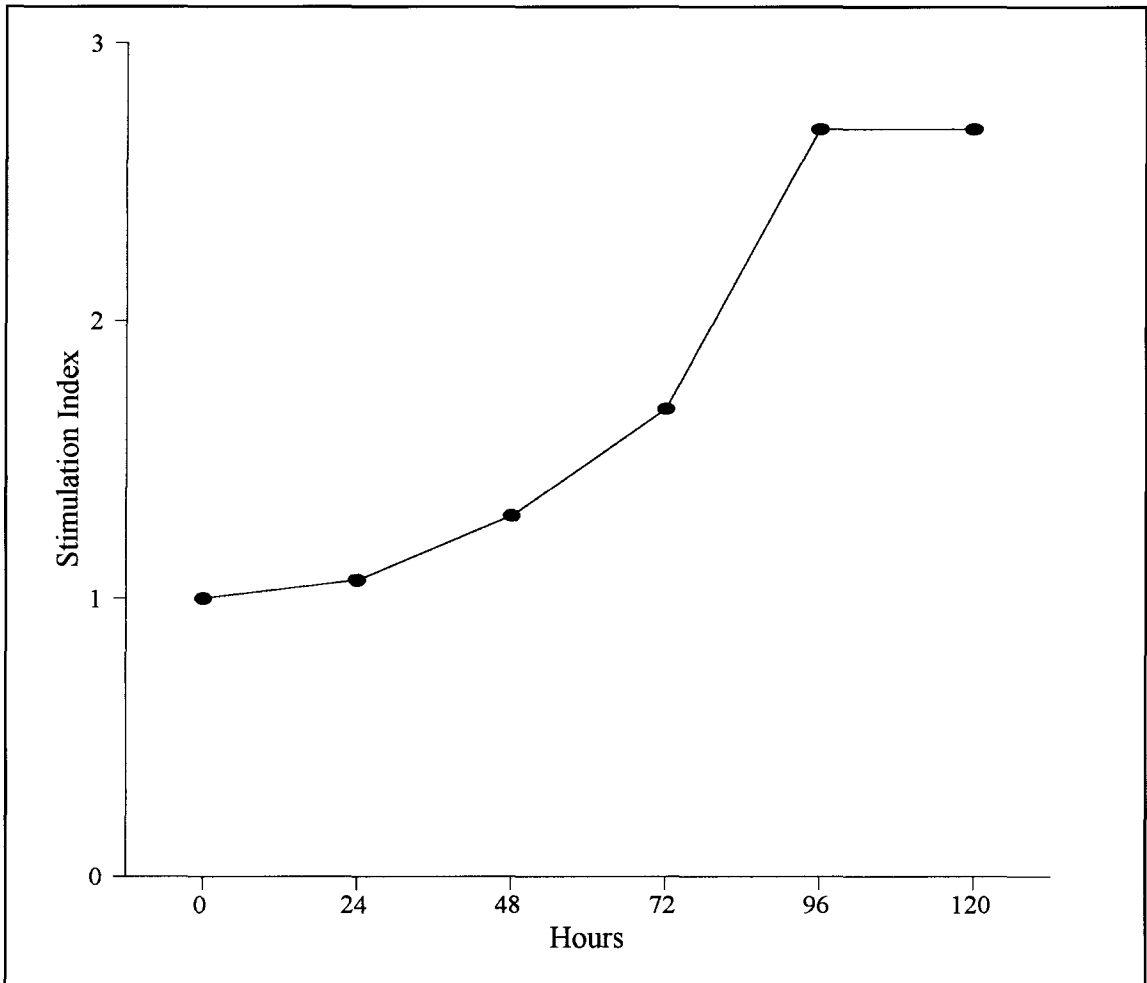


Fig. 1. Time dependent modulation of membrane-bound CD26 peptidase activity by human PBMC following activation with 1µg/ml PHA (single representative donor).

membranes abound in these receptors, and endothelial ligands are expressed by extra-oral nodal as well as tonsillar endothelium. Many of these ligands are also expressed on the membrane of lymphoid and myeloid cells, and mediate cell-cell contact (5,6,7,25-30).

These endothelial ligands constitute the large family of cell adhesion molecules. Some of the representatives of this group of molecules are the intercellular adhesion molecules (ICAM)-1,2 and 3 (CD54, CD102 and CD50, respectively), the vascular adhesion molecule (VCAM)-1 (CD106), and

the mucosal addressin cell adhesion molecule (MAdCAM)-1 (CD\*, not yet assigned). The expression of adhesion molecules is finely regulated and plays a crucial role in the modulation of the migration and recirculation of T and B lymphocytes during inflammation and immune surveillance of the oral cavity. Additional relevant endothelial membrane molecules have been identified, which take part in haptotaxis and which interact with the extracellular matrix of the peri-oral lymphoid tissues. Examples include fibronectin, collagen and laminin (25-27).

Specific membrane receptors empower lymphocytes to interact with these adhesion molecules. These receptors, collectively called integrins, represent a versatile family of leukocyte membrane receptors, and their expression is rapidly and exquisitely modulated in response to the immunophysiological milieu and the state of activation and of maturation of the lymphocyte (5,25,27,28). Other lymphocyte membrane receptors that do not belong to the integrin family also participate in the haptotactic phenomenon by interacting with adhesion molecules. In point of fact, it would suffice to mention the membrane receptor CD26, a membrane-bound extracellular serine peptidase (dipeptidylpeptidase IV; EC.3.4.14.5), whose expression and activity rises considerably several hours following T lymphocyte activation (*Fig. 1*). The role of CD26 includes inactivating substance P by removal of the first two NH<sub>2</sub>-terminal amino acids, and its substrate affinities include fibronectin (31,32). CD26-expressing activated lymphocytes within the lymphoid tissue parenchyma appear to be cells capable of modulating, perhaps even neutralizing certain neuropeptidergic-lymphoid and epithelial-lymphoid interactions.

Integrins share a common molecular structure in that they all are composed of noncovalently joined  $\alpha$  and  $\beta$  chains. To date, five principal integrins have been identified to play a crucial role in specific endothelial-lymphoid interaction: lymphocyte function-associated antigen (LFA)-1 (CD11a= $\alpha$ L/CD18= $\beta$ 2), whose ligand is ICAM-2 (CD102); Mac-1 (C3R) (CD11b= $\alpha$ M/CD18), whose ligands are ICAM-1 (CD54), iC3b, fibrinogen and Factor X; very late antigen (VLA)-4 (CD49d= $\alpha$ 4/CD29= $\beta$ 1), whose ligands are VCAM-1 (CD106) and fibronectin; and lymphocyte-Peyer's patch adhesion molecule (LPAM)-1 (CD49d/CD\*= $\beta$ 7), whose ligands are MAdCAM-1, VCAM-1 and fibronectin in the Peyer's patches. Additional critical integrins include P150,95

(CD11c= $\alpha$ X/CD18), which binds to iC3b and fibronectin; VLA-1 (CD49a= $\alpha$ 1/CD29), VLA-2 (CD49b= $\alpha$ 2/CD29) and VLA-3 (CD49c= $\alpha$ 3/CD29), which recognize collagen and laminin; VLA-5 (CD49e= $\alpha$ 5/CD29), which binds fibronectin and thrombospondin; VLA-6 (CD49f= $\alpha$ 6/CD29), which recognizes exclusively laminin; and the human mucosal lymphocyte (HML)-1 (CD103= $\alpha$ E/CD\*= $\beta$ 7) that binds to ICAM-1. It is important to reiterate at this juncture that the bond between T cell integrins (LFA-1, VLA-1-6, p150,95, Mac-1, HLM and LPAM-1) and endothelial cell matrix (laminin, fibronectin, collagen) or endothelial cell adhesion molecules (ICAM-1-3, VCAM-1, MAdCAM-1) is stabilized and strengthened by integrin modulating factor (IMF)-1 and other cytokines and chemokines produced at, or proximal to, the lymphocyte endothelium attachment site (5,7,25-28).

Lymphocytes home to extra-oral lymph nodes and tonsillar lymphoid tissues in a specific manner that is determined in large part by the homing receptor they express. These receptors respond to chemo- and haptotactic stimuli, as well as to the state of activation and maturation of the lymphocytes. As naive CD4 and CD8 T cells (CD45RA+) circulate and home, they establish initial contact with the high endothelium of the microvasculature by means of the L-selectin receptor (CD62L, lymphocyte endothelial cell adhesion molecule [LECAM]-1). CD62L recognizes with avidity two mucins expressed by nodal high endothelial venules (HEVs): the soluble glycosylation-dependent cell adhesion molecule (GlyCAM)-1, and the endothelial cell membrane-bound CD34 moiety. CD62L also recognizes and avidly binds to MAdCAM-1 expressed by mucosal high endothelial venules. Cytokine-activated endothelium expresses an endothelium-leukocyte adhesion molecule (ELAM)-1, which shows substantial structural homology to L-selectin and which is now referred to as E-selectin (CD62E). Under certain

conditions, CD62L and CD62E can serve as a reciprocal receptor-ligand pair (5,7,25-28). Whether GlyCAM-1 acts as a soluble antagonist of CD62L binding to membrane-bound CD34, MAdCAM-1 and CD62E is unclear. Further, while it is clear that tonsillar endothelium is characterized by high endothelial venules, it remains to be determined whether it expresses predominantly the nodal (CD34) or the mucosal ligand (MAdCAM-1) to CD62L.

A myriad of additional associated interactions contribute to lymphocyte-endothelium adhesion. For instance, cross-linking of the common leukocyte antigen, CD45, induces a rapid agglutination of lymphocytes and their avid sticking to surfaces coated with ICAM-1 (33). CD44, a complex molecule found in several isoforms with different affinities and specificities, is expressed by lymphocytes and many other cell types, and binds to hyaluronic acid moieties on cell membranes (6,28). Soluble forms of integrins, adhesion molecules, selectins and their ligands have recently been detected, as predicted (29), in several pathological conditions (7). Whereas it may be argued that the soluble form of these molecules serves to impede immune cell agglutination, the presence of these soluble forms of adhesion ligands in microvessels probably contributes to the immunophysiological regulation of lymphocyte-endothelium adhesion and transendothelial migration, and thus contributes to the modulation of immune surveillance processes. It will be important to fully characterize the molecular mechanisms of these events, particularly in the context of immune surveillance of the oral cavity.

In summary, recirculation of T and B lymphocytes in peri-oral lymphoid tissue involves several distinct events. Initially, the lymphocyte, subject to the forces of blood flow and of chemotaxis approaches its anatomical site of destination. As the blood capillary progressively decreases in diameter, as the force of the flow diminishes and as the chemotactic force increases, the chances that

the lymphocyte will come in contact with the vascular endothelium increases. Hence, haptotaxis can commence. When the lymphocyte reaches the region of the microvasculature endowed with high endothelium in the extra-oral nodes and in the tonsils, and if the migratory cell expresses CD62L, a loose CD62L-ligand bond is established that serves to anchor the cell at this site. This induces transmembrane signaling events that initiate to activate the lymphocyte. Consequently, cytokines and other factors are produced that strengthen chemotaxis. Expression of integrins by the activated lymphocytes and of adhesion molecules by the endothelium ensues. These molecules consolidate the lymphocyte-endothelium bond. Among the early events in activated lymphocytes is the loss of CD62L; thus prompt expression of integrins and adhesion molecules is crucial for lymphocyte-endothelial adhesion to continue successfully. The last step in this adhesion/migration cascade to and through nodal and tonsillar lymphoid tissue is the transendothelial migration between the cells forming the high endothelium (26,27,34,35).

Lymphocyte migration to extra-oral lymphoid tissues occurs by means of interactions between the lymphocytes and the high endothelium of the extra-oral lymph nodes. Migration to sub-mucosal, salivary and gingival lymphoid tissues occurs predominantly through postcapillary venules, in a manner similar to that which takes place in other sites of the mucosal immune system (e.g., intestinal lamina propria) (4). Lymphocyte migration to tonsillar lymphoid tissue occurs through tonsillar high endothelium.

Tonsillar high endothelium develops as early as the 14th gestational week and is observed predominantly in the parafollicular regions of the tonsils throughout the life span. Tonsillar high endothelium is rich in ICAM-1. Immunochemical data demonstrates that ICAM-1 expression is primarily confined to the vascular endothelium, in close proximity to LFA-1-expressing lymphocytes. Indeed, tonsillar ICAM-1 immunoreactivity is noted



on the luminal and lateral surfaces of the high endothelium, but not on the abluminal surface supported by the basal lamina. Lymphocytes that express LFA-1 are also found on ICAM-1-positive high endothelium located in reticulated crypts. The tonsillar capsule contains a significant additional number of lymphocytes positive for the VLAs. T cells (CD3+) are observed in the tonsils to express LFA-1, and to be closely juxtaposed to ICAM-1+ endothelial cells within the regions endowed with high endothelium (24,36-38). The lymphoendothelial symbiosis in the tonsils thus reflects that observed in peripheral lymph nodes, and demonstrates that specific patterns are at play at these sites that regulate T cell migration and recirculation through well-defined high endothelium in human tonsillar tissue. This arrangement argues against a random array of lymphocytes and endothelial cells at these anatomical sites.

As in the instance of T cell migration through the high endothelium of extra-oral lymph nodes, the state of maturation and of activation of the lymphocyte is crucial in the determination of its migratory properties through the tonsillar high endothelium. It is noteworthy that CD4+ T cells ( $\approx 40\%$ ) predominate in human tonsillar lymphoid tissue compared to CD8+ T cells ( $\approx 5\%$ ), with B cells representing the highest proportion of lymphocytes ( $\approx 55\%$ ). Human tonsillar germinal centers contain CD4+ T cells that do not express CD45RA or CD62L, but bear the memory phenotype (CD45R0), and are capable of promoting B cell maturation and proliferation. Naive CD4+ T cells that express CD45RA+ and the few remaining CD4+ T cells that still express CD62L+ within the human tonsils are found in the tonsillar interfollicular areas (11,14). Whereas rats do not technically have tonsils, they do show tonsil-equivalent lymphoid tissue, which resembles human tonsillar material by most histochemical and functional criteria. This rodent tissue is characterized by a T cell/B cell ratio of 0.7 and a CD4+ T cell/CD8+ T

cell ratio of 5, which are similar to those observed in human tonsils and reported above. These data, taken together with the observation that rat T lymphocytes adhere equally well to rat tonsillar high endothelium under experimental conditions compared to peripheral lymph node high endothelium (11,39) indicate that the use of human tonsils removed at operation could provide excellent and readily available experimental tissue to study human lymphocyte adhesion to and through high endothelium.

#### *RELEVANCE FOR ALCOHOL AND DRUG ABUSERS*

Epidemiologic studies indicate a prevalence of cancer of the oro- and nasopharynx among individuals who jointly abuse alcohol and nicotine (40). Indeed, recent data indicate that chronic alcohol consumption is a key factor in the development of pharyngeal cancer, independently from exposure to tobacco (41,42). The mechanisms of alcohol- and alcohol/nicotine-mediated carcinogenesis must be elucidated in order to develop effective means of immunogenetic therapeutic interventions for the afflicted patients. Understanding the fundamental processes of lymphocyte recirculation in peri-oral lymphoid organs should shed light about immune surveillance of oral cancers because increasing evidence demonstrates that lymphocyte migration shares many molecular commonalities with the metastatic process (28,35), and because alcohol seems to alter metastatic events (35,43). Taken together these lines of evidence are particularly relevant to the increasing number of HIV-seropositive individuals, many of whom have a variety of oral diseases including aggressive tumors. These patients often drink alcoholic beverages, often in conjunction with smoking, unaware of the potential ill effects of this combination (44-46).

Chronic exposure to alcohol alters immune defense mechanisms and results in abnormal endothelial histopathology. Liver

sinusoidal capillarization, characterized by defenestration, development of a basement membrane, collagenization and diffuse fibrosis of the interstitium commonly follow chronic alcohol intake. Cytopathological sequelae of alcohol are also noteworthy in that some endothelial cells appear to be blocked at the G<sub>0</sub>/G<sub>1</sub> interphase (45,47).

Substantial information supports that alcohol blunts cell-mediated immune processes (30,35,43,45), including lymphocyte recirculation. Alcohol abusers demonstrate decreased number of circulating CD8+ T cells and CD4+ T cells that bear the CD62L homing receptor (48). Whether alcohol induces selective toxicity to CD62L T cell populations, or whether it induces alteration of the migration properties of these cells to high endothelium (49) has not, however, been fully resolved. In fact, a dearth of research has specifically addressed the question of whether or not alcohol and other addictive drugs act synergistically to blunt cellular mediated immune surveillance of the oral cavity by altering specifically the migratory properties of T cells in peri-oral lymphoid tissue.

Current studies from our laboratory have begun to shed light on these issues. We have isolated 95-99% enriched CD4+CD62L+ T cells by means of a specific protocol (30,50,51), and then treated the recovered CD4+CD62L+ T cells with varied concentrations ethanol for 24-48 h, and monitored the cultures by dual color by flow cytometry. We found an average 35% decrease in CD62L+ T cells (25% drop specifically in CD4+CD62L+ T cells) after incubation at 0.2% but not lower concentrations of ethanol, suggesting alcohol-mediated toxicity. Parallel experiments have begun to test the ability of CD4+CD62L+ T cells to adhere to and to migrate through normal human tonsillar endothelial cells (CRL-7175, ATCC). Toxic concentrations of ethanol also appear to impede the ability of lymphocytes to adhere to these endothelial monolayers.

Taken together, these lines of evidence support the hypothesis that alcohol alters the

homing properties of CD4+ T cells. This phenomenon could be one mechanism by which alcohol impairs immune surveillance of the oral cavity and thus favors the onset of oral tumors and other diseases. Ongoing studies are designed to elucidate the molecular mechanisms for these outcomes, and to characterize the effect of other addictive drugs, such as nicotine and cocaine often used in conjunction with alcohol. The ultimate goal is to attain a better understanding of the normal physiology of oral immune surveillance, and alcohol immunotoxicity, and to develop effective modes of immune therapies for pathological manifestations within the oral cavity.

#### ACKNOWLEDGMENT

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