Endothelial Cell-Mediated Antigen Presentation*

David R. Johnson

Clinical Immunology Branch, DAIT, NIAID, NIH, Bethesda, MD, USA

Key Words

Endothelial cells · Immune tolerance · Transplantation tolerance · Transgenes

Summary

Immune recognition of endothelial cells (ECs) has been implicated in a number of vascular diseases. Although ECs have been shown to stimulate T lymphocytes in vitro, these conditions were not physiological, and it remained unclear what occurred in vivo. Antigen presentation by ECs in vivo was tested using transgenic mice that express a protein antigen (β -galactosidase, β -gal) exclusively in their ECs. This transgenic approach avoided potentially inflammatory manipulations. Transgenic mice responded to immunization with the antigen (protein or DNA) with strong B and T cell-mediated immunity. No effects of the immune response on the vessels in vivo could be detected, and ECs continued to express β -gal. Skin grafts from transgenic mice onto nontransgenic mice suffered a type of vascular rejection, strongly suggesting that specific lymphocytes in hosts recognize and respond against β -gal+ ECs in the skin grafts. However, heart transplants containing β -gal+ ECs were accepted indefinitely by nontransgenic hosts. Moreover, skin grafts with β -gal+ ECs were accepted onto hosts with hearts containing β -gal+ ECs, suggesting that the immune system had recognized and learned to tolerate the heart ECs. The implications for transplantation and persistent infection are discussed.

Schlüsselwörter

Endothelzellen · Immuntoleranz · Transplantationstoleranz · Transgene Tiere

Zusammenfassung

Die Erkennung von Endothelzellen durch das Immunsystem ist in eine Reihe vaskulärer Erkrankungen involviert. Endothelzellen können in vitro T-Lymphozyten stimulieren. In-vitro-Bedingungen entsprechen jedoch nicht physiologischen Bedingungen, und es ist unklar, ob ein solcher Effekt auch in vivo existiert. Zur Untersuchung der Frage der Antigenpräsentation durch Endothelzellen unter in vivo Bedingungen wurden transgene Mäuse eingesetzt, die ein Proteinantigen (β -galactosidase, β -gal) ausschließlich in den Endothelzellen exprimieren. Dieses Modell vermeidet experimentell notwendige Manipulationen, die zu inflammatorischen Reaktionen führen könnten. Die transgenen Mäuse reagierten auf die Antigen-Immunisierung (Protein oder DNA) mit einer ausgeprägten B- und T-Zell-vermittelten Immunität. In vivo konnte kein Effekt der Immunantwort auf die Gefäße nachgewiesen werden, und die Endothelzellen exprimierten weiterhin β -gal. Hauttransplantate transgener Mäuse auf Wildtyp-Mäuse zeigten eine Abstoßungsreaktion, die darauf hinweist, dass antigenspezifische Lymphozyten des Transplantatempfängers die β -gal exprimierenden Endothelzellen des Hauttransplantats erkennen und darauf reagieren. Im Gegensatz dazu wurden Herztransplantate, die β -gal-exprimierende Endothelzellen aufweisen, von Wildtyp-Mäusen auch über einen längeren Zeitraum toleriert. Hauttransplantate, die β -gal-exprimierende Endothelzellen aufweisen, wurden von Wildtyp-Mäusen toleriert, die zuvor ein für β -gal-exprimierende Endothelzellen positives Herztransplantat erhalten hatten. Diese Beobachtung weist darauf hin, dass das Immunsystem die β -gal-exprimierenden Endothelzellen des Herztransplantates erkennt und Toleranzmechanismen entwickelt hat. Die vorliegende Übersichtsarbeit diskutiert die Bedeutung dieser Untersuchungsserie für Transplantationen und persistierende Infektionen.

All opinions expressed herein are those of the author and do not necessarily represent the position of the United States government.

Introduction

Health requires an immune system that protects against infectious microbes while tolerating the many different tissues in the body. Analysis of immune tolerance usually focuses on the location of tissue antigens – within the central immune system or in the periphery – a distinction that overlooks the interface between these compartments. This interface is formed by the endothelium, a single cell layer that lines all lymphatic and blood vessels. Endothelial cells (ECs) are extraordinarily thin to optimize gas exchange between the blood and tissues. ECs also play key roles in recruiting leukocytes from the circulation into the peripheral tissues and from the lymph into lymphoid organs.

The immune system regulates infectious EC pathogens, including viruses, such as cytomegalovirus (CMV), and intracellular bacteria, such as *Rickettsia* and *Chlamydia pneumoniae*. Chronic CMV infection of ECs is controlled by T lymphocytes. The infection can spread when host immunity is weakened or suppressed by disease or chemotherapy, or when infected tissue is transplanted to naïve recipients. Chronic stimulation of the immune system by persistent infections also might be important in the development of vasculitis, arteriosclerosis and diseases of uncertain pathogenesis.

Despite the importance of ECs in immune-mediated vascular diseases, the details of immune recognition of ECs remain obscure. The ability of ECs to stimulate lymphocytes has been analyzed by culturing isolated cell populations in vitro, by histology, and by manipulation of cells in situ. Recently, this question was approached using transgenic mice genetically engineered to express a new protein only within their ECs [1]. This approach avoided potential artifacts introduced by cell isolation and culture. By relying on genetically regulated expression, the approach also avoided potentially proinflammatory interventions in situ. Upon analysis of the antigen-specific lymphocytes in these transgenic mice, it was concluded that EC proteins are recognized and tolerated by the immune system. General aspects of EC-lymphocyte interactions have been reviewed recently [2]. Here, the focus is on the background and experimental findings from studies of immune recognition of EC antigens.

Principles

Endothelium

The vasculature pervades all tissues, bringing erythrocytes bearing oxygen and carbon dioxide close to all cells of the body. The supply and demand for oxygen is defined by the concentration of oxygen available in the atmosphere and the high metabolic rate of vertebrates. The density of vessels depends on the low solubility and diffusion of oxygen through the aqueous bodily fluids as well as physical constraints, such

as resistance to flow, which increases dramatically as vessels narrow. Together, these factors result in most cells being within a few cell widths of a blood vessel.

Gas exchange occurs across capillaries which are formed by ECs, a basal lamina and a variable number connective tissue cells called pericytes that wrap processes around the basilar side of ECs and help maintain the capillary wall. Although gas exchange probably accounts for most vascularization, nutrients, hormones and other signaling molecules also transit the endothelium in both directions in vesicles. Liver and kidney capillary ECs allow macromolecules to pass freely through specialized regions called fenestrae. In contrast, brain ECs constitute the blood-brain barrier which can severely limit molecular exchange. Large vessels have additional layers of smooth muscle cells and elastic bands which contribute to maintaining blood pressure.

Endothelium actively participates in the movement of leukocytes between the blood and tissues. ECs respond to inflammatory cytokines and chemokines present in the blood (lumen) or within the parenchymal tissues. Newly synthesized or activated proteins expressed on the EC surface bind specific subsets of leukocytes through a series of steps involving rolling and adhesion mediated by selectins, integrins and enzymes [3]. The normally tight junctions between ECs can quickly and transiently open to permit the movement of selected leukocytes while maintaining a barrier to serum proteins. Most recruited leukocytes are not antigen-specific. Although it is not known whether antigen-specific lymphocytes can be selectively recruited by ECs, it is worthwhile considering the potential efficiency of such a system. If ECs could present antigen to circulating lymphocytes, then the vasculature might function as a high-throughput device for selecting antigen-specific lymphocytes from the bloodstream. This would be valuable for bringing to bear the very small number of antigen-specific lymphocytes rapidly to fight infection.

Immune System

Lymphocytes

Lymphocytes are responsible for the antigen specificity of the immune response. Antigen receptors are expressed on the 3 types of lymphocytes: T (thymus-dependent), B (bone marrow-derived) and NK (natural killer). B cell antigen receptors are immunoglobulins which bind antigen directly. Secreted immunoglobulins (antibodies) can kill cells by fixing complement or by directing nonspecific killer leukocytes. T cell antigen receptors (TCRs) recognize antigenic peptides bound to cell surface molecules encoded within the MHC – called HLA in humans and H-2 in mice (fig. 1).

T cells that express the cluster-of-differentiation-4 molecule on their surface (CD4+) usually recognize antigenic peptides held by class II MHC molecules, which are expressed constitutively on few cells (fig. 1). MHC class II expression is inducible

Fig. 1. Lymphocytes recognize different forms of antigen. B cells and antibodies recognize intact proteins or larger fragments. T cells recognize short peptides held on the cell surface by MHC class I or II molecules.

on most cells by IFN- γ , a product of activated T cells. CD4⁺ T cells help B cells make antibodies, and they regulate other immune responses [4]. CD8⁺ T cells usually recognize antigenic peptides held by class I MHC molecules, which are expressed constitutively on nearly all nucleated cells [5]. Cytotoxic T lymphocytes (CTL) are CD8⁺ and can kill infected or foreign cells. Both helper T (Th) cells and CTL can secrete a wide range of regulatory cytokines, including the autocrine growth factor interleukin-2 (IL-2) and the differentiation factors IL-4, IFN- γ and TNF- α . Whereas T cells are activated when recognizing a specific complex of peptide and MHC molecule, the same complex can inhibit NK cells.

Antigen Presenting Cells

T cell activation requires accessory cells called antigen presenting cells (APCs) [6]. APCs degrade protein antigens, producing peptides that bind MHC molecules. The dependence of T cells on APCs explains the attention paid to understanding the details of MHC expression by ECs [7]. ECs of many vascular beds are among the few cells in the body that express high levels of MHC class I and class II molecules in humans [8, 9]. In contrast, mouse cardiac ECs express constitutively MHC class I but not class II molecules [10], which may underlie their inability to directly stimulate T cells specific for donor MHC [11]. Other murine ECs do express class II MHC molecules constitutively, with the level of expression depending upon the tissue [12]. It should also be noted that for both human and mouse ECs, expression above a basal level of MHC class I requires cytokines, particularly IFN- γ [13]. Therefore, MHC expression by ECs varies among the different vascular beds and changes depending upon the environmental and pathogenic stimuli.

MHC class I and II molecules screen different pools of proteins for potentially antigenic peptides. MHC class I molecules bind peptides, 9–11 amino acids long, derived from intracellular proteins (typically, synthesized endogenously) [14, 15]. MHC class II molecules bind peptides derived from extracellular proteins that are endocytosed and degraded in the lysosome. MHC class II molecules bind longer peptides at a variety of cellular locations, including the cell surface and in specialized organelles of some APCs.

Cellular antigens can be presented directly or indirectly (also known as cross-priming) [16]. The distinction is clearest in the

Table 1. Costimulator molecules expressed by EC

Costimulator	EC	T cell
CD80 (B7-1)	no	CD28
CD86 (B7-2)	no	CD152 (CTLA-4)
CD40	yes	CD154 (CD40L)
CD11a/CD18 (LFA-1)	yes	CD54 (ICAM-1)
CD58 (LFA-3)	yes	CD2

case of transplantation. Direct presentation occurs when specific T cells recognize peptides bound to graft (donor) MHC molecules. Indirect presentation occurs when specific T cells recognize peptides derived from the graft on recipient (host) MHC molecules. Similarly, tissue-specific peptides may be recognized directly on cells of the tissue or indirectly, following phagocytosis, on other APCs.

Dendritic cells (DCs) are the premier, ‘professional’ APCs [17]. Highly phagocytic, immature DCs are found in many tissues where they are called interstitial DCs. Interstitial DCs in the skin are called Langerhans cells. DCs can efficiently present peptides derived from phagocytosed proteins on MHC class I molecules. DC maturation is triggered by a number of relatively nonspecific microbial stimulators of innate immunity, such as lipopolysaccharide or unmethylated CpG. Mature DCs move to the draining lymphatics, phagocytose less, express more MHC molecules, secrete IL-12 and TNF- α , and provide better costimulation (see next).

Costimulation

In addition to antigen stimulation, T cells require a number of other signals to proliferate and mature. Costimulation is the term given to these additional signals [18]. Cells vary widely in their ability to provide costimulation and act as APCs [19]. Although costimulation of CD4⁺ Th cells is better characterized, resting CD8⁺ T cells also require costimulation for efficient development into effector and memory CTL [20].

The best-characterized costimulation pathway involves CD28 molecules on T cells binding to CD80 and CD86 molecules (B7-1 and B7-2) on APCs. CD86 expression is increased on mature DCs. CD80 is expressed by DCs, monocytes and activated B cells [21]. CD152 (cytotoxic T lymphocyte antigen-4, CTLA-4) is an alternative T cell ligand for CD80 that reduces T cell activation [22, 23]. TCR signaling and CD28/B7 costimulation induces CD152 expression, suggesting that CD152 acts in a negative feedback system to dampen the immune response. Many memory Th cells require CD80 stimulation to avoid anergy [24]. However, CD80 is not necessary for allospecific Th cells [25] and not sufficient [26]. Moreover, CD28 knockout mice mount protective immune responses to vesicular stomatitis virus and lymphocytic choriomeningitis virus (LCMV) in vivo [27].

Costimulation is also mediated by molecules related to B7 and

Table 2. Mechanisms for avoiding autoimmunity

Form	Antigen recognition	Cause	Consequence
Ignorance	none	sequestration	no effect on lymphocytes
Tolerance	central peripheral	thymic expression inadequate costimulation	clonal deletion deletion, anergy, or regulation

the TNF receptor (TNFR). B7h, B7-H1 and B7-DC do not bind CD28 or CD152. B7h (also called ICOS-L for inducible costimulator ligand) binds ICOS on activated T cells. B7-H1 and B7-DC (also called programmed death ligand-1 and -2) bind PD-1 on T cells. The murine orthologs include the B7-related protein-1 (B7RP-1) and CD28-related protein-1 (ICOS). TNFR-related costimulators include CD27, 4-1BB (CD137), OX40 (CD134), HVEM, CD30, GITR and TL-1 [28]. ECs are known to express some of these B7-/TNFR-related and other costimulators, though their activities are not clearly defined [2].

Activated Th cells express CD40 ligand (CD40L, also called CD154), which binds the costimulator CD40 expressed on ECs, DCs and B cells. CD40-CD40L interactions activate ECs, and help B cells to mature [29, 30]. Similarly, Th cells activate DCs which are then able to stimulate CTL maturation [31–33]. Activating antibodies against CD40 replace Th cells in promoting chronic rejection of MHC class II-mismatched heart allografts [34]. Host CD40L deficiency prolongs the survival of fully mismatched heart allografts although chronic rejection develops eventually.

Costimulation might regulate the development of Th cell subpopulations. Th type 1 (Th1) cells produce IFN- γ and TNF- α , mediate defense against infectious disease and transplant rejection, and can cause autoimmunity. Th2 cells express IL-4, IL-5 and IL-10, which mediate allergic reactions and modulate Th1 responses.

Tolerance

The importance of controlling immune responses so that they do not threaten the host was appreciated from nearly the beginning of the science of immunology. Bordet demonstrated in 1899 that antibodies and complement could lyse erythrocytes. However, it was soon realized that although antibodies could easily be raised against foreign proteins and cells, it was relatively difficult to raise antibodies against self-proteins. This observation led Ehrlich to hypothesize that the immune system resists mounting self-destructive responses ('horror auto-toxicus') [35]. The cellular, molecular and systemic details of immunological self-tolerance are still under investigation.

The immune system avoids self-destruction (autoimmunity) through passive and active processes mediated largely by T cells. T cells arise from the bone marrow and mature in the thymus. Most T cells entering the thymus die from neglect or

overstimulation. Survival requires that their TCR binds peptide-MHC complexes (positive selection) but not too strongly (negative selection). Thus, T cells expressing TCRs with moderate avidity for MHC molecules bearing self-peptides are released from the thymus. However, mature T cells may encounter peptides from tissue specific proteins that were not present in the thymus. Here, the strength of costimulation provided by the APCs determines the outcome of T cell antigen recognition. Insufficient costimulation can lead to T cell deletion by induction of anergy (paralysis) or apoptosis (programmed cell death) [24, 36].

T cells ignore or tolerate self-proteins (table 2). Some potential antigens are sequestered in organs such as the eye, testes and brain. Other peripheral antigens, such as insulin, are expressed in the thymus and presented to developing T cells due to the transcriptional activator protein AIRE (autoimmune regulator) [37]. Immature T cells that recognize self-proteins within the thymus are deleted. T lymphocytes encountering antigens expressed by certain tissues may be killed in the periphery, maintaining tolerance. The pro-apoptotic molecule Fas ligand (FasL) is expressed on the surface of cells in the retina and testes [38]. T cells recognizing antigen on these tissues may be triggered to undergo Fas-mediated apoptosis. ECs do not express FasL and are not known to induce T cell apoptosis.

T cells integrate both stimulatory and inhibitory signals from APCs. Autoimmune diseases can be ameliorated or worsened by blocking costimulation, depending on the target and timing. CD152 (CTLA-4) reduces T cell activation. CD152 knockout mice suffer polyclonal CD4⁺ T cell proliferation and die within 4 weeks of birth [39]. Adding CD152⁺ lymphocytes to CD152 knockout mice protects them from lymphoproliferative disease, demonstrating that the CD152⁺ cells are dominant. In addition, antibody cross-linking of CD152 induces the secretion of the immunosuppressive cytokine TGF- β [40]. CD152⁺ T cells may act through overall suppression or by redirecting the response to a Th1 pattern which encourages cellular immunity. Thus, CD152⁺ cells may be critical in establishing tolerance to proteins expressed in the periphery.

Regulatory (Suppressor) T Cells

About 20 years ago, suppressor T cells were widely invoked to explain many immune phenomena. This ended when no genes were found in a predicted suppressor control locus (I-J), and

none of the putative antigen-specific suppressor factors were cloned. Interest in suppression was renewed by studies using sophisticated cellular and animal systems. For the historical reasons mentioned, suppressor T cells have reappeared in the literature under the name 'regulatory T cells' (T reg).

Two well-characterized T reg phenotypes are thought to mediate tolerance of peripheral antigens, both are CD4⁺ and express CD25, the high affinity T cell growth factor receptor [41]. One type of T reg expresses CD152 (described above), and the other expresses high levels of CD5 and low levels of CD45RB/RC, a marker of T cell maturation [42]. CD4⁺ T cells depleted of the CD25⁺ CD5^{high} or CD45RB/RC^{low} subpopulations produce a wide range of autoimmune diseases in mice, including thyroiditis, gastritis and insulin-dependent diabetes mellitus. These diseases are caused by autoantibodies and cell-mediated immunity [42]. The development of T reg depends on a Foxp3, a transcription factor and potential 'master regulator' of T reg [43].

Antigen Presentation by Endothelial Cells

The ability of ECs to present antigens to T cells is critical for inflammatory responses within the vasculature, and ECs are also anatomically positioned to present antigens to circulating T cells into the periphery [44, 45].

Lymphocyte Traffic

Lymphocytes that have never recognized antigen are called naïve. Naïve lymphocytes that recognize antigen and receive costimulation (T cells) or help (B cells) are stimulated to mature and proliferate, generating effector and memory cells. Isoforms of the phosphatase CD45 distinguish T cell populations: naïve T cells are CD45RA⁺ while memory T cells are CD45RO⁺ in humans or CD45RB⁺ in mice. Although activated T cells do not often mutate their antigen receptors, self-reactive T cell receptors can be 'revised' in the periphery [46]. Naïve B cells express immunoglobulin M (IgM) and IgD antigen receptors on their surface. Activated B cells switch to IgG isotypes and also mutate the antigen binding site, resulting in some clones with increased affinity. Thus, a memory immune response in mice is characterized by CD45RB⁺ T cells and a high titer, high affinity, largely IgG isotype antiserum.

Naïve and memory T cells have different patterns of circulation [47]. Naïve T cells circulate largely in the blood and lymphoid tissues. Naïve T cells first encounter antigens presented by DCs within lymph nodes. Antigens reach the lymph nodes upon draining from the peripheral tissues or upon being brought by tissue DCs. Naïve T cells and a subset of memory T cells express the chemokine receptor CCR7 and the cell adhesion molecule L-selectin, which direct these cells to the lymph nodes [48]. Antigen recognition by naïve T cells triggers their proliferation and maturation, forming a population of memory T cells. Memory T cells circulate through the pe-

ripheral tissues, returning by the draining afferent lymph to the lymph node and reentering the blood through the thoracic duct. The expanded population of memory T cells is more likely to encounter antigen during their transits through the peripheral tissues. Under flow conditions, memory, but not naïve, T cells adhere lightly to ECs ('roll') mediated by E- and P-selectins and VCAM-1 [49]. Therefore, the response of memory T cells to antigen presented by ECs may be particularly important in the secondary response.

Vascular Bed ECs

Lymphocytes home to the intestines, inflamed skin or other sites through recognition of tissue-specific molecules expressed by ECs. The CC chemokine TARC is expressed on the venules of chronically inflamed skin but not intestines. Many memory T cells express the TARC receptor, CCR4. TARC binding triggers the arrest of lymphocytes rolling on endothelium, activating integrin-dependent adhesion of skin (but not intestinal) homing of memory T cells to ICAM-1 under physiological flow [50].

ECs derived from different vascular beds respond differently to cytokines. TNF induces ICAM-1 expression on both arterial and venous adult human iliac ECs. However, VCAM-1 is expressed only on TNF-activated venous ECs which consequently bind VLA-4⁺ (CD49a/CD29) T cells better [51]. Both, iliac arterial and venous ECs, costimulate IL-2 and IFN- γ secretion, but not IL-4 secretion, by human peripheral blood T cells or CD4⁺ T cell clones [52].

T Cell Recruitment

Antigen-specific lymphocytes induce, accelerate, amplify and prolong inflammation. Although many of the molecules involved in leukocyte recruitment have been identified, the recruitment of lymphocytes that are specific for the inflammatory stimulus is poorly understood.

The TCR contributes only negligibly to binding APC. Although some TCRs have significant affinity for a given MHC/peptide structure (dissociation constant (K_d) ~ 10 μ mol/l, [53]), few MHC molecules are likely to contain the particular peptide. However, the rapid dissociation of TCR-MHC complexes may produce a 'serial triggering' of many TCRs by a single MHC-peptide structure [54, 55]. TCR recognition of the MHC/peptide structure can trigger an increase in the affinities of T cell integrins [56]. Cross-linking TCR-associated CD3 molecules on resting human T cells, which mimics antigen recognition, transiently increases the affinities of LFA-1 and VLA integrins [57, 58]. Normal lymphocyte recirculation to lymphoid tissues is blocked by antibody specific for CD18 (LFA-1) [59]. Therefore, activated T cells cannot be used in studies of antigen-specific recruitment because selectivity may depend on antigen-recognition triggering an integrin switch to high affinity [60].

Although ECs acting as APCs for specific T cells is an attractive method for bringing specific T cells to the inflammation,

alternative mechanisms are reasonable and supported experimentally. Instead of presenting antigens, ECs at sites of inflammation may only select T cells already activated in the lymph nodes. Small, resting T cells harvested from the lymph migrate to lymph nodes but not to an inflammation, whereas those isolated from the blood migrate to an inflammation and not lymph nodes. Similarly, activated T cells migrate to an inflammation and not lymph nodes [61]. Iliac ECs or human umbilical vein ECs (HUVEC) activated with TNF do not co-stimulate T cells better despite significant increases in cell adhesion molecule expression [52]. EC activation actually decreases CD8+ T cell costimulation and transendothelial migration in vitro [62].

Medical Observations

Many important vascular diseases are caused or complicated by antigen-specific immune responses against ECs. Lymphocyte-mediated inflammation is central to many cardiovascular diseases [63, 64]. Atherosclerosis and chronic graft rejection may be specialized forms of chronic inflammation in which monocytes and T cells attack ECs [65]. Acute disorders of the blood, including disseminated intravascular coagulation and hemorrhage, can be initiated by lymphocytes-secreting antibodies and cytokines that activate or injure ECs. Controlling these diseases will require a detailed understanding of the cellular and molecular events underlying the antigen-specific recognition of ECs by lymphocytes.

Inflammation

The 3 classic signs of inflammation (redness, swelling and warmth) result from blood vessel dilation which reduces resistance and increases blood volume. Vessel dilation slows blood flow, thereby promoting leukocyte interactions with ECs. ECs also respond to inflammatory cytokines by changing their expression of surface molecules in a characteristic pattern, increasing adhesion for particular leukocytes. Leukocyte diapedesis is divided into 4 steps: tethered rolling, triggering, tight adhesion and movement through the endothelium out of the lumen [66].

Chemoattractant cytokines (chemokines) are released during inflammation. Alloreactive CD8+ T cells secrete the chemokine RANTES (recruited on activation of normal T cells and secreted) which binds inflamed ECs and mediates monocyte adhesion under flow conditions [67]. Chemokines also activate integrin cell adhesion molecules which require a conformational change for high-affinity binding [68]. Integrins important for leukocyte-EC adhesion include LFA-1, which binds ICAM-1 and ICAM-2 on ECs, and VLA-4, which binds fibronectin and VCAM-1 on cytokine-activated human ECs [51]. The chemokines SDF-1 and MIP-3 β induce binding of

most circulating lymphocytes through CCR7. MIP-3 α triggers the integrin-dependent adhesion of memory, but not naïve, T cells [69].

Prior exposure to infectious diseases and parasites can be tested by a cutaneous, inflammatory delayed type hypersensitivity (DTH) reaction which depends on memory T cells. DTH is quicker (approximately 24 h) and stronger in sensitized individuals [70]. Injecting protein antigens leads to CD4+ T cell accumulation. Intact proteins do not stimulate CD8+ T cells efficiently. However, specific CD8+ T cells accumulate after injection of peptides or infection [71]. DTH is blocked by antibodies specific for either LFA-1 or CD44 (pgp-1) [59]. CD44 binds to an extracellular matrix component that accumulates at sites of inflammation. P-selectin and E-selectin on ECs also help recruit Th1 cells into inflamed tissues [45]. Additional 'bystander' lymphocytes without specificity for the sensitizing protein are usually also recruited [72].

Infections

Immune responses against persistent endothelial cell infections, such as CMV or *C. pneumoniae*, have been suggested to initiate or exacerbate atherosclerosis, heart disease and other vascular diseases [73, 74]. Epidemiology suggests that both microbes are risk factors, and both are found in atherosclerotic plaques [73, 75]. Thus, although not established, an immune component of atherosclerosis is strongly suspected [76–78]. The development of atherosclerosis can be accelerated by a memory T cell response against vascular cells [79]. These studies used apolipoprotein E-deficient (apoE knockout) mice that were maintained on a high-fat diet which produces mild atherosclerosis. The disease became significantly worse, however, when an immune response was generated against the vascular smooth muscle cells. Similarly, infection with murine CMV and *C. pneumoniae* increase lesion size in apoE knockout mice [74]. The protective effect of antibiotics in a rabbit model of atherosclerosis triggered by *C. pneumoniae* supports a role for this microbe in vascular disease [80].

Immune responses against infected ECs cause a number of diseases, including hemorrhagic fever, Chagas' disease, Kawasaki's disease and systemic vasculitis. *Rickettsia*, *Trypanosoma cruzi* and Hantaviruses infect ECs [81–83]. EC injury from direct and immune mechanisms results in EC dysfunction, with reduced vascular tone (hypotension) and increased vascular permeability (vascular leak). Disseminated intravascular coagulation also occurs in some patients.

Transplantation

Organ transplantation is the definitive therapy for most forms of end stage organ disease. One-year graft survival approaches 90% for some organs. Heart transplantation is the only suc-

cessful treatment for diseases that result in heart failure [84]. Kidney transplantation accounts for over half of all transplant procedures. Transplantation is the preferred treatment for end-stage renal disease which results from many common diseases including diabetes and glomerulonephritis. Lung transplantations are performed for chronic obstructive lung disease, cystic fibrosis, idiopathic pulmonary fibrosis and pulmonary hypertension. The primary obstacle to therapeutic transplantation of vascularized organs and tissues is chronic rejection. Approximately half of all grafts fail within 5 years due to a process of intimal thickening and sclerosis of the vessels termed chronic graft rejection or transplant accelerated atherosclerosis. For patients who survive the first year after heart or lung transplantation, the leading cause of death is chronic rejection [85]. The development of chronic rejection is poorly understood [86]. ECs are targets and perhaps important stimulators of the immune response against transplanted organs. The extent of subendothelial lymphocyte infiltration indicates the severity of transplanted organ rejection [87]. Chronic rejection is almost certainly initiated by the host immune response against different (allo) MHC molecules expressed on donor cells. Class I MHC molecules are highly expressed by ECs which could serve as initiators or targets of alloreactive host T cells ('injury hypothesis') [88]. Class II MHC molecules, also strong stimulators of an alloresponse, may be induced by IFN- γ secreted by alloreactive host T cells [77, 89].

Vascular Rejection

Vascular rejection summarizes the importance of blood supply in successful transplant engraftment. After the loss of blood supply during surgery (ischemia), reperfusion of the graft occurs when host blood vessels are surgically or naturally connected to donor vessels. Murine allogeneic skin grafts stimulate specific host CD8⁺ T cells to make chemokines and cytokines [67]. In response to host T cells, graft ECs express MHC class II molecules [90]. Allogeneic class II MHC molecules probably stimulate host CD4⁺ helper T cells which amplify the rejection response.

Intimal thickening induced by transplantation is reduced 50% in CD4 knockout mice, demonstrating that helper T cells contribute to the development of chronic graft rejection in this model system [91]. Chronic graft rejection develops normally in class I MHC-deficient (β_2m -knockout) mice, but these mice develop CTL despite lacking normal class I MHC molecules [92–94]. Aberrant recognition may contribute to the observation that grafts from β_2m -knockout mice actually develop atherosclerosis faster than grafts from normal mice [95]. In the absence of CD4⁺ T cells, CD40 signaling and CD8⁺ T cells also lead to graft rejection [34]. In the absence of CD28 costimulation, NK cells can mediate cardiac graft rejection [96]. These are complex model systems in which a large number of immune reactions are induced simultaneously against a variety of antigens expressed by a number of different cell types.

Similar questions are explored below in a much simpler model system: a single protein antigen (β -gal) expressed by a single cell type (EC).

Research Approaches

Several different approaches have been taken to determining whether ECs are good APCs. Antigen recognition can produce tolerance or aggression, depending on the precise interactions between T cells and APCs. Several groups have shown that ECs from different vascular beds are capable of performing certain APC functions in vitro [52, 62, 97, 98]. However, EC activities as APCs in vivo are poorly defined.

ECs Are 'Semiprofessional' APCs in vitro

ECs have been termed 'semiprofessional' APCs because they costimulate certain T cell responses in vitro and are thought stimulate alloresponses in vivo [62, 99–102]. HUVEC costimulate, along with a mitogenic stimulus, T cell proliferation and IL-2 secretion [19, 103]. In contrast, vascular smooth muscle cells do not provide costimulation [52]. Costimulation occurs through LFA3-CD2 and additional, undefined pathway(s) but not CD28-CD80/CD86 [52, 104]. Human iliac artery and vein ECs also costimulate Th1 clones to produce more IFN- γ . Th2 clones are not costimulated to produce more IL-4 [52].

HUVEC only costimulate memory T cells in vitro [105–107]. They also stimulate a lower frequency of alloreactive CTL than do matched lymphoblast lines [108]. Moreover, many CTL raised against ECs in vitro kill only ECs, and persistently express the normally transient activation markers CD40L and CD69 [109]. Murine ECs also costimulate T cell proliferation and cytokine secretion in vitro [110], although no murine homologue of LFA-3 has been reported. Murine lung microvascular ECs express CD80, yet they also costimulate only memory T cells in vitro [62]. Thus, both murine and human ECs are capable of some APC activities in vitro.

Testing ECs as APCs in vivo

Cells often change when they are cultured in vitro. For example, smooth muscle cells express different collagens in vivo and in vitro [111]. ECs also express extraordinarily high levels of MHC class I molecules in vivo but not in vitro [8 and unpublished observations]. Although HUVEC are readily obtained, easily cultured and widely studied, they are fetal and perhaps programmed for senescence [112]. Unfortunately, it is difficult to obtain or culture adult ECs from different vascular beds. Growth control and probably many other normal cellular functions depend on tissue structure and integrity [113]. In addition, some cell adhesion molecules function only in fluid

Table 3. Mouse strains used in the study of antigen presentation by EC.

Strain	Background	Transgene	Expression	Reference
FVB	FVB (wild type)	none	none	[118]
VWF-lacZ	FVB	lacZ	β-gal in microvessel EC of heart and brain	[116]
Tie2-lacZ	FVB	lacZ	β-gal in all EC, particularly large vessels	[117]

flow. For these reasons, more physiological conditions were sought to test antigen presentation by ECs.

Transgenic mice are ideal for investigating antigen presentation by particular cell types. Transgene expression avoids the inflammation caused by other means of gene targeting, such as transfection or infection, and the changes that occur when cells are cultured. Tissue specific gene promoters have been used to test the immune consequences of a restricted expression of the transgene lacZ which encodes *Escherichia coli* β-galactosidase (β-gal). B lymphocytes expressing β-gal are tolerated by β-gal-specific lymphocytes. When these tolerant mice are immunized with β-gal, only low-titer, low-affinity antisera are raised [114]. In contrast, astrocytes expressing β-gal are ignored. β-gal immunization of these mice raises a strong humoral and cellular response that leads to neuropathology [115]. Similarly, smooth muscle cells expressing β-gal are ignored [79]. However, immunization with peptide pulsed DCs leads to myocarditis and arteritis. Especially intriguing in this study was the finding that immunity amplified and prolonged arteritis in atherosclerosis-prone apoE knockout mice.

Transgenic Mouse Model

There are 2 transgenic mouse lines that express the β-gal-encoding *E. coli* lacZ gene exclusively in their ECs (table 3). In the VWF-lacZ transgenic line, a portion of the promoter and first intron of the gene encoding von Willebrand factor (VWF), a procoagulant EC protein, regulates the expression of β-gal [116]. In the Tie2-lacZ transgenic line, a portion of the promoter and first intron of the gene encoding Tie2, the receptor for the vascular differentiation factor angiopoietin-1, regulates the expression of β-gal [117].

Characterization of the Transgenic Mice

Both strains of transgenic mice were generated on the FVB background and were never bred with any other strain. All 3 strains are identical except for the transgene (congenic). To keep the strains as genetically similar as possible, hemizygous transgenics were maintained by backcrossing to the FVB parental strain [118]. Transgenic offspring were identified from PCR analysis of genomic DNA obtained from ear punches.

The immune system can respond against small differences between self- and non-self-proteins. Thus, it was important that the β-gal proteins were identical. The transgenes in both

strains came from the plasmid pSDKLacZpA, i.e. from the same source. The PCR products from both transgenic strains were sequenced and confirmed as lacZ by comparison to a reference sequence (GenBank accession U00096). LacZ transcripts in the organs of transgenic mice were analyzed by RT-PCR [7]. As expected, lacZ mRNA was detected in the heart tissue of VWF-lacZ mice, but not in tissue from the lungs or spleen. LacZ transcripts were detected in all organs tested from the Tie2-lacZ mice. No lacZ transcripts were detected in any organ of the nontransgenic FVB mice. Control samples in which reverse transcription was omitted confirmed that genomic DNA contamination was not responsible for the products.

Expression of β-gal protein in tissues was analyzed by staining with X-gal (5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside) which is a chromogenic substrate for β-gal [119]. A faint blue precipitate indicated weak β-gal activity in ECs of cardiac microvessels in the VWF-lacZ transgenic mice. No other VWF-lacZ organs showed activity. In contrast, ECs in all organs tested from Tie2-lacZ mice showed activity, and large vessels in the heart stained very strongly. Therefore, the β-gal expression patterns were exactly as reported by the groups that generated these transgenic mice.

B Cell Responses

1. Protein immunization: Tolerance to the β-gal self-protein was tested by measuring the immune response elicited upon immunization with protein or DNA. It was expected, normal FVB mice but not tolerant animals would generate a strong, mature response. After immunization with β-gal protein in complete Freund's adjuvant, both FVB and transgenic mice raised strong antisera. To determine the nature of the T cell help provided to the B cells, the immunoglobulin isotypes of the anti-β-gal antibodies were analyzed to determine the type of T help provided the B cells. Th1 cells increase IgG2a isotype responses while Th2 cells increase IgG1 isotype responses. Therefore, the ratio of IgG1 to IgG2a isotype antibodies provides a measure of the Th activities stimulated by the antigen. No differences in the ratios of IgG1 vs. IgG2a antibodies are observed, suggesting that similar patterns of Th activities are stimulated in FVB and transgenic mice.

Tolerance is often most marked on lymphocytes possessing high-affinity antigen receptors. High-affinity antibodies can be measured in inhibition assays because they dissociate more

slowly than low-affinity antibodies, so they remain inhibited longer upon pre-incubation with soluble antigen. Such assays showed that antisera from FVB and transgenic mice display similar affinities for β -gal, demonstrating that B cells making high-affinity antibodies were not tolerant in the transgenic mice.

2. *DNA immunization*: An immune response can be elicited by injecting genes encoding a protein directly into the skin of mice. This is done using a device that shoots DNA-coated gold particles directly into cells in the living animal [120, 121]. Such genetic immunization stimulates cellular immune responses more than antibody responses [122], perhaps in part because the MHC class I antigen presentation pathway is stimulated by the endogenously synthesized antigens. Cellular immunity also may be stimulated due to particular DNA sequence motifs [123]. All mice, normal and transgenic, that were immunized with the gene gun formed high-titer antisera against β -gal. The antisera had equivalent affinities. This route of immunization was potent and highly reproducible using the same preparations of DNA-gold particles. The system seemed attractive for testing co-expression of cytokines, chemokines, costimulator molecules, etc. Unfortunately, the system proved to be unacceptably variable when using a different expression vectors (personal observation).

3. *Bacterial antigens*: Proteins expressed in bacteria are efficiently processed in the MHC class I antigen presentation pathway [124]. A published protocol whereby mice are efficiently immunized with heat-killed bacteria was tested [125]. DH5 bacteria harboring the plasmid pUC19 were induced to express β -gal, then heat-killed by autoclaving (less aggressive heating did not kill all bacteria). Notably, heat denatured β -gal is reported to be a good antigen for inducing CTL [126]. Unfortunately, the immunized mice produced negligible antisera against β -gal.

T Cell Responses

Proliferation assays demonstrated no differences between normal and transgenic T cells responding against β -gal protein. Popliteal lymph node cells were prepared 6 days after protein immunization in base of the tail [127]. T cells from normal and transgenic mice showed equivalent dose-responses and kinetics [1]. Both CD4⁺ and CD8⁺ T cells increased and blocking antibodies specific for MHC class I and II molecules reduced proliferation, indicating that CD4⁺ and CD8⁺ T cells were responding.

Blood Vessel Morphometry

To evaluate potential blood vessel remodeling initiated by immune stimulation, multiple blood vessels were harvested, fixed, sectioned and analyzed morphometrically. The infrarenal artery, ascending aorta, coronary artery, carotid artery and iliac artery, as well as vessels in the heart, liver, lung and spleen, were examined. No changes were detected in the lumen, intima, media or vessel size (defined by the external

elastic lamina). There were no histological abnormalities noted. It was possible that ECs at sites of injury would be particularly susceptible to an immune response. To test this, mouse femoral arteries were injured and denuded of ECs using a wire. Upon regrowth of the endothelium, no differences were observed between the vessels from immunized and nonimmunized Tie2-lacZ mice (Jun Yu, personal communication).

Transplantation

Transplantation is a classic immunological tool that can detect even small differences between donors and recipients. The survival of skin and heart grafts transplanted between normal FVB and transgenic mice was tested.

1. *Skin transplantation*: Skin was transplanted between transgenic and nontransgenic littermates from (FVB/NJ $\times$ Tie2-lacZ hemizyote) crosses. Mice were 6–8 weeks old and sex matched to avoid responses against H-Y. Full thickness skin grafts (1 cm²) with small portions of subcutaneous fat were excised and a comparably sized piece of littermate skin was placed on the defect and stapled in place. The donor skins were reversed anterior-posterior so that the graft could be distinguished by the direction of fur growth. Serum samples collected 6 weeks after skin transplantation were tested for antibodies specific for β -gal. Normal mice that received transgenic skins (+ $\rightarrow$ -) generated antibodies against β -gal. Transgenic mice receiving either normal skin or transgenic skin (- $\rightarrow$ + or + $\rightarrow$ +) did not generate antisera against β -gal. These control experiments demonstrated that immune response was specific for the β -gal in the skin graft ECs and not due to the injury itself. The IgG2a isotype dominated the antibody response against the transgenic skin, which is consistent with the primarily cellular response (Th1-type) of this immunoglobulin isotype [128].

2. *Tolerance*: There were 2 possible results following the grafting of Tie2-lacZ skin onto VWF-lacZ mice: either the VWF-lacZ hosts would tolerate the graft because they tolerated their own β -gal⁺ (cardiac) ECs or they would reject the graft and perhaps initiate an immune response against their own ECs. Tie2-lacZ skin grafted on the VWF-lacZ recipients healed much more quickly than on the FVB recipients. Reversed fur growth was noted on many of the VWF-lacZ recipients but not the FVB recipients. Of the mice receiving Tie2-lacZ skin grafts, all the FVB recipients and none of the VWF-lacZ recipients generated β -gal specific antisera (fig. 2). Moreover, β -gal⁺ ECs were lost from the grafts on FVB recipients but retained in the grafts on VWF-lacZ recipients. These differences could not have been due to variations in the donor tissue, because single donors were used for transplants onto mice from both control and experimental groups.

3. *Heart tolerance*: Cardiovascular diseases may be caused by immune responses against ECs. To test the recognition of cardiac ECs by lymphocytes, transgenic hearts were transplanted into the abdomen, beneath the kidney of normal recipient

Fig. 2. Skin grafts containing β -gal+ ECs induce immune responses on normal but not transgenic mice.

mice (heterotopic, infrarenal). The cardiac arteries of the heart grafts are connected to the host blood supply by an end-to-side anastomosis to the aorta. The blood is returned through the pulmonary artery of the graft to the vena cava of the host. The transplanted heart can be felt beating in the host abdomen. Technical success, defined as a beating heart 3 days after transplant, was over 80%. Most Tie2-lacZ and VWF-lacZ hearts transplanted into normal FVB mice survived for more than 5 months (Tie2-lacZ $\rightarrow$ FVB or VWF-lacZ $\rightarrow$ FVB, fig. 3). These were harvested by perfusion/fixation and analyzed for β -gal expression. All transgenic hearts continued to express β -gal in their ECs.

Lymphocytes

The antiserum and T cell proliferation studies demonstrate that β -gal-responsive lymphocytes were not deleted or anergized in Tie2-lacZ or VWF-lacZ mice. However, it was possible that transgenic mice delete lymphocyte subsets that could otherwise mediate autoimmune responses. To investigate this, mature lymphocytes (splenocytes) were transferred from non-transgenic FVB to transgenic mice. Transgenic recipients showed no evidence of autoimmunity (weight loss, scruffy appearance). T cells from the draining lymph nodes of immunized mice were also transferred to transgenic mice. Again, there was no evidence of autoimmunity over 6 months, and the ECs in mice of both transgenic strains continued to express β -gal.

Conclusions

Resting ECs are efficient APCs, capable of presenting intracellular proteins to the immune system. A strong immune response involving both T cells and B cells can be stimulated, but the response can avoid damaging ECs expressing the same proteins. These observations suggest that immune response, and tolerance, is dependent on the context in which the antigen is presented.

The difference in the responses between heart and skin graft ECs might be attributable to vascular bed heterogeneity or

Fig. 3. Heart transplants containing β -gal+ ECs are tolerated by normal and transgenic mice.

differences in the experimental procedures, especially ischemia. In heart transplantation, the donor heart vessels are connected directly to the host blood supply, thereby reducing ischemic injury and activation of the ECs [64]. Although skin grafts are thin and survive the interruption in blood supply, these grafts undergo prolonged hypoxia which activates ECs. Alternatively, the vascular beds are fundamentally different, such that dermal ECs are more immunogenic than cardiac ECs. Transgenic mice do not respond against (i.e., they tolerate) autologous skin grafts, demonstrating that ischemia alone is not sufficient to make the graft immunogenic.

To determine whether the immune system recognized or ignored cardiac ECs, the ability of VWF-lacZ mice to respond against Tie2-lacZ skin grafts was tested. Rejection of ECs in Tie2-lacZ skin transplanted onto VWF-lacZ mice would have clearly demonstrated vascular bed differences. Instead, the Tie2-lacZ skin grafts were accepted by VWF-lacZ mice, demonstrating unambiguously that the immune system recognized and was tolerized by proteins expressed by intact ECs (fig. 3).

The rules for tolerance remain obscure. Several lines of inquiry might help clarify them, though many more insightful ideas will be needed to fully reveal the system. Although no vascular injury was detected, it remains an important medical concern. Can a strong immune stimulation break tolerance of the transplanted heart ECs? Tolerance of ECs might be broken by transplanting skin from Tie2-lacZ mice onto FVB recipients of Tie2- or VWF-lacZ heart transplants. (This is the preferred order of transplants because the younger mice fair better upon heart transplantation.) If the transplanted skin stimulates an immune response, the response may also recognize the heart ECs and provoke rejection. It is also possible that the heart transplantation will establish tolerance. An alternative approach would be to use a pathogen, such as LCMV encoding β -gal, to stimulate a strong immune response against ECs. It is possible that subtle changes were present in the vasculature of mice undergoing an immune response to EC proteins. A more sensitive analysis could measure the expression of cell adhesion molecules associated with inflammation, such ICAM-1, on capillary ECs.

Peptides are very useful probes of T cell recognition because they do not depend on antigen processing – they can load directly onto MHC proteins on the cell surface. Peptides were not employed in the experiments described here, because the immune dominant peptide of β -gal in the host MHC is not known. Peptides should be employed to stimulate stronger T responses without transplantation [129]. Peptides could also help measure fine differences in immune recognition of β -gal between normal and transgenic mice.

Vascular diseases may be exacerbated by immune responses against ECs. ECs are important targets and perhaps stimula-

tors of the immune response against infections and allogeneic transplanted organs. The observations reviewed here suggest that the immune system may also restrain responses against ECs. Such restraint may avoid excessive thrombogenesis and explain why ECs are subject to persistent infections. Thus, the immune system may sacrifice pathogen clearance for homeostasis. If understood better, these mechanisms might be manipulated intentionally to reduce vascular injury, improve transplantation outcome, or even permit targeting ECs for gene therapy [130].

References

- Rothermel A, Wang Y, Schechner J, Mook-Kanamori B, Aird W, Pober J, Tellides G, Johnson D: Endothelial cells present antigens in vivo. *BMC Immunol* 2004;5(1):5.
- Choi J, Enis D, Koh K, Shiao S, Pober J: T lymphocyte-endothelial cell interactions. *Annu Rev Immunol* 2004;22:683–709.
- Salmi M, Jalkanen S: Cell-surface enzymes in control of leukocyte trafficking. *Nat Rev Immunol* 2005;5(10):760–771.
- Cooper M, Lawton A 3rd: Primary Immune Deficiency Diseases (chapter 307); in Fauci A, et al (eds): *Principles of Internal Medicine*. New York, McGraw-Hill, 1998, pp 1783–1791.
- Germain R: MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 1994;76(2):287–299.
- Janeway C Jr, Bottomly K: Signals and signs for lymphocyte activation. *Cell* 1994;76(2):275–285.
- Johnson DR, Mook-Kanamori B: Dependence of elevated HLA class I molecule expression on increased heavy chain, light chain (β 2m), TAP, tapasin, and peptide. *J Biol Chem* 2000;275(22):16643–16649.
- Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ: The detailed distribution of HLA-A, B, C antigens in normal human organs. *Transplantation* 1984;38(3):287–292.
- Daar AS, Fuggle SV, Fabre JW, Ting A, Morris PJ: The detailed distribution of MHC Class II antigens in normal human organs. *Transplantation* 1984;38(3):293–298.
- Choo J, Seebach J, Niekleit V, Shimizu A, Lei H, Sachs D, Madsen J: Species differences in the expression of major histocompatibility complex class II antigens on coronary artery endothelium: implications for cell-mediated xenoreactivity. *Transplantation* 1997;64(9):1315–1322.
- Kreisel D, Krasinskas A, Krupnick A, Gelman A, Balsara K, Popma S, Riha M, Rosengard A, Turka L, Rosengard B: Vascular endothelium does not activate CD4+ direct allorecognition in graft rejection. *J Immunol* 2004;173(5):3027–3034.
- Karasin A, Macvilay S, Hart M, Fabry Z: Murine endothelia do not express MHC class II I-Ealpha subunit and differentially regulate I-Aalpha expression along the vascular tree. *Endothelium* 1998;6(2):83–93.
- Goes N, Urmson J, Hobart M, Halloran P: The unique role of interferon- γ in the regulation of MHC expression on arterial endothelium. *Transplantation* 1996;62:1889–1894.
- Schumacher TN, de Bruijn ML, Vernie LN, Kast WM, Melief CJ, Neeffes JJ, Ploegh HL: Peptide selection by MHC class I molecules. *Nature* 1991;350(6320):703–706.
- Townsend A, Rothbard J, Gotch F, Bahadur B, Wraith D, McMichael A: The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 1986;44(959):959–968.
- Benichou G, Valujskikh A, Heeger PS: Contributions of direct and indirect T cell alloreactivity during allograft rejection in mice. *J Immunol* 1999;162(1):352–358.
- Fuchs EJ, Matzinger P: B cells turn off virgin but not memory T cells. *Science* 1992;258(5085):1156–1159.
- Unanue E: Macrophages, antigen-presenting cells, and the phenomena of antigen handling and presentation; in Paul W (ed): *Fundamental Immunology*. New York, Raven Press, 1993, pp 111–144.
- Ashida E, Johnson A, Lipsky P: human endothelial cell-lymphocyte interaction: endothelial cells function as accessory cells necessary for mitogen-induced human T lymphocyte activation in vitro. *J Clin Invest* 1981;67:1490–1499.
- Liu Y, Wenger R, Zhao M, Nielsen P: Distinct costimulatory molecules are required for the induction of effector and memory cytotoxic T lymphocytes. *J Exp Med* 1997;185:251–262.
- Schwartz R: Co-stimulation of T-lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* 1992;71:1065–1068.
- Gribben J, Freeman G, Boussiotis V, Rennett P, Jellis C, Greenfield E, Barber M, Restivo V Jr, Ke X, Gray G, Nadler L: CTLA4 mediates antigen-specific apoptosis of human T cells. *Proc Natl Acad Sci U S A* 1995;92:811–815.
- Boise L, Noel P, Thompson C: CD28 and apoptosis. *Curr Opin Immunol* 1995;7(5):620–625.
- Gimmi C, Freeman G, Gribben J, Gray G, Nadler L: Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc Natl Acad Sci U S A* 1993;90:6586–6590.
- Boussiotis V, Freeman G, Gray G, Gribben J, Nadler L: B7 but not intercellular adhesion molecule-1 costimulation prevents the induction of human alloantigen-specific tolerance. *J Exp Med* 1993;178(5):1753–1763.
- Lombardi G, Sidhu S, Dodi T, Batchelor R, Lechler R: Failure of correlation between B7 expression and activation of interleukin-2-secreting T cells. *Eur J Immunol* 1994;24(3):523–530.
- Shahinian A, Pfeffer K, Lee K, Kundig T, Kishihara K, Wakeham A, Kawai K, Ohashi P, Thompson C, Mak T: Differential T cell costimulatory requirements in CD28-deficient mice. *Science* 1993;261(5121):609–612.
- Watts T: TNF/TNFR family members in costimulation of T cell responses. *Annu Rev Immunol* 2005;23:23–68.
- Karmann K, Min W, Fanslow WC, Pober JS: Activation and homologous desensitization of human endothelial cells by CD40 ligand, tumor necrosis factor, and interleukin 1. *J Exp Med* 1996;184(1):173–182.
- Korthauer U, Graf D, Mages HW, Briere F, Padayachee M, Malcolm S, Ugazio AG, Notarangelo LD, Levinsky RJ, Kroczeck RA: Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature* 1993;361(6412):539–541.
- Ridge JP, di Rosa F, Matzinger P: A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* 1998;393(6684):474–478.
- Schoenberger SP, Toes RE, van der Voort EI, Of-fringa R, Melief CJ: T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 1998;393(6684):480–483.
- Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR: Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 1998;393(6684):478–80.
- Fischbein MP, Ardehali A, Yun J, Schoenberger S, Laks H, Irie Y, Dempsey P, Cheng G, Fishbein MC, Bonavida B: CD40 signaling replaces CD4+ lymphocytes and its blocking prevents chronic rejection of heart transplants. *J Immunol* 2000;165(12):7316–7322.
- Silverstein AM: The history of immunology; in Paul WE (ed): *Fundamental Immunology*. Philadelphia, Lippincott-Raven, 1999, pp 19–35.
- Perillo N, Pace K, Seilhamer J, Baum L: Apoptosis of T cells mediated by galectin-1. *Nature* 1995;378:736–739.
- Anderson M, Venanzi E, Chen Z, Berzins S, Benoist C, Mathis D: The cellular mechanism of Aire control of T cell tolerance. *Immunity* 2005;23(2):227–239.
- Strand S, Hofmann WJ, Hug H, Muller M, Otto G, Strand D, Mariani SM, Stremmel W, Krammer PH, Galle PR: Lymphocyte apoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumor cells – a mechanism of immune evasion? [see comments]. *Nat Med* 1996;2(12):1361–1366.
- Chambers C, Sullivan T, Allison J: Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. *Immunity* 1997;7(6):885–895.
- Chen W, Jin W, Wahl S: Engagement of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) induces transforming growth factor beta (TGF-beta) production by murine CD4(+) T cells. *J Exp Med* 1998;188(10):1849–1857.
- Chatenoud L, Salomon B, Bluestone J: Suppressor T cells – they're back and critical for regulation of autoimmunity! *Immunol Rev* 2001;182:149–163.

- 42 Sakaguchi S: Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 2000;101(5):455–458.
- 43 Khattri R, Cox T, Yasayko S, Ramsdell F: An essential role for Scurfin in CD4⁺CD25⁺ T regulatory cells. *Nat Immunol* 2003;4(4):337–342.
- 44 Pober JS, Cotran RS: The role of endothelial cells in inflammation. *Transplantation* 1990;50(4):537–544.
- 45 Austrup F, Vestweber D, Borges E, Löhning M, Bräuer R, Herz U, Renz H, Hallmann R, Scheffold A, Radbruch A, Hamann A: P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. *Nature* 1997;385:81–83.
- 46 Fink PJ, McMahan CJ: Lymphocytes rearrange, edit and revise their antigen receptors to be useful yet safe. *Immunol Today* 2000;21(11):561–566.
- 47 Mackay CR, Marston WL, Dudler L: Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 1990;171(3):801–817.
- 48 Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A: Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999;401(6754):708–712.
- 49 Lichtman AH, Ding H, Henault L, Vachino G, Camphausen R, Cumming D, Luscinskas FW: CD45RA-RO⁺ (memory) but not CD45RA-RO⁻ (naïve) T cells roll efficiently on E- and P-selectin and vascular cell adhesion molecule-1 under flow. *J Immunol* 1997;158(8):3640–3650.
- 50 Campbell JJ, Haraldsen G, Pan J, Rottman J, Qin S, Ponath P, Andrew DP, Warne R, Ruffing N, Kassam N, Wu L, Butcher EC: The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 1999;400(6746):776–780.
- 51 Hauser I, Johnson D, Madri J: Differential induction of VCAM-1 on human iliac venous and arterial endothelial cells and its role in adhesion. *J Immunol* 1993;151:5172–5185.
- 52 Johnson D, Hauser I, Voll R, Emmrich F: Arterial and venular endothelial cell costimulation of cytokine secretion by human T cell clones. *J Leuk Biol* 1998;63:612–619.
- 53 Corr M, Slanetz A, Boyd L, Jelonek M, Khilko S, Al-Ramadi B, Kim Y, Maher S, Bothwell A, Margulies D: T cell receptor-MHC class I peptide interactions: affinity, kinetics, and specificity. *Science* 1994;265:946–949.
- 54 Vallittu S, Müller S, Cella M, Padovan E, Lanzavecchia A: Serial triggering of many T-cell receptors by a few peptide-MHC complexes. *Nature* 1995;375:148–151.
- 55 Viola A, Lanzavecchia A: T cell activation determined by T cell receptor number and tunable thresholds. *Science* 1996;273(5271):104–106.
- 56 Hauenberger D, Klominek J, Bergstrom SE, Sundqvist KG: T lymphocyte migration: The influence of interactions via adhesion molecules, the T cell receptor, and cytokines. *Crit Rev Immunol* 1995;15:285–316.
- 57 Dustin M, Springer T: T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 1989;341:619–624.
- 58 Shimizu Y, van Sevringer G, Horgan K, Shaw S: Regulated expression and binding of three VLA (β1) integrin receptors on T cells. *Nature* 1990;345:250–253.
- 59 Camp R, Scheynius A, Johansson C, Puré E: CD44 is necessary for optimal contact allergic response but is not required for normal leukocyte extravasation. *J Exp Med* 1993;178:497–507.
- 60 Ebnet K, Kaldjian E, Anderson A, Shaw S: Orchestrated information transfer underlying leukocyte endothelial interactions. *Ann Rev Immunol* 1996;14:155–177.
- 61 Issekutz T, Webster D, Stoltz J: Lymphocyte recruitment in vaccinia virus-induced cutaneous delayed-type hypersensitivity. *Immunology* 1986;58:87–94.
- 62 Marelli-Berg FM, Scott D, Bartok I, Peek E, Dyson J, Lechler RI: Activated murine endothelial cells have reduced immunogenicity for CD8⁺ T cells: a mechanism of immunoregulation? *J Immunol* 2000;165(8):4182–4189.
- 63 Sullivan GW, Sarembock IJ, Linden J: The role of inflammation in vascular diseases. *J Leukoc Biol* 2000;67(5):591–602.
- 64 Albelda S, Smith C, Ward P: Adhesion molecules and inflammatory injury. *FASEB J* 1994;8:504–512.
- 65 Ross R: Atherosclerosis – an inflammatory disease. *N Engl J Med* 1999;340(2):115–126.
- 66 Springer T: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;76:301–304.
- 67 Koga S, Novick AC, Toma H, Fairchild RL: CD8⁺ T cells produce RANTES during acute rejection of murine allogeneic skin grafts. *Transplantation* 1999;67(6):854–864.
- 68 Hynes R: Integrins: versatility, modulation, and signalling in cell adhesion. *Cell* 1992;69:11–25.
- 69 Campbell J, Hedrick J, Zlotnik A, Siani M, Thompson D, Butcher E: Chemokines and the arrest of lymphocytes rolling under flow conditions. *Science* 1998;279:381–384.
- 70 Issekutz TB, Stoltz JM, van der Meide P: Lymphocyte recruitment in delayed type hypersensitivity, the role of IFN-γ. *J Immunol* 1988;140(2928):2928–2993.
- 71 Kündig T, Althage A, Hengartner H, Zinkernagel R: Skin test to assess virus-specific cytotoxic T-cell activity. *Proc Natl Acad Sci USA* 1992;89:7757–7761.
- 72 Müller U, Steinhoff U, Reis L, Hemmi S, Pavlovic J, Zinkernagel R, Aguet M: Functional role of type I and type II interferons in antiviral defense. *Science* 1994;264:1928–1931.
- 73 de Boer OJ, van der Wal AC, Becker AE: Atherosclerosis, inflammation, and infection. *J Pathol* 2000;190(3):237–243.
- 74 Burnett MS, Gaydos CA, Madico GE, Glad SM, Paigen B, Quinn TC, Epstein SE: Atherosclerosis in apoE knockout mice infected with multiple pathogens. *J Infect Dis* 2001;183(2):226–231.
- 75 Ouchi K, Fujii B, Kudo S, Shirai M, Yamashita K, Gondo T, Ishihara T, Ito H, Nakazawa T: *Chlamydia pneumoniae* in atherosclerotic and nonatherosclerotic tissue. *J Infect Dis* 2000;181:S441–443.
- 76 Schoen F: Blood vessels; in Cotran R, Kumar V, Robbins S (eds): *Pathologic Basis of Disease*, 5th ed. Philadelphia, WB Saunders, 1994, pp 467–516.
- 77 Salomon RN, Hughes CC, Schoen FJ, Payne DD, Pober JS, Libby P: Human coronary transplantation-associated arteriosclerosis. Evidence for a chronic immune reaction to activated graft endothelial cells. *Am J Pathol* 1991;138(4):791–798.
- 78 Hansson G: Immune responses in atherosclerosis; in Hansson G, Libby P (eds): *Immune Functions of the Vessel Wall*. Amsterdam, Harwood Academic Press, 1996, pp 143–158.
- 79 Ludewig B, Freigang S, Jaggi M, Kurrer MO, Pei YC, Vlk L, Odermatt B, Zinkernagel RM, Hengartner H: Linking immune-mediated arterial inflammation and cholesterol-induced atherosclerosis in a transgenic mouse model. *Proc Natl Acad Sci U S A* 2000;97(23):12752–12757.
- 80 Fong I: Antibiotics effects in a rabbit model of *Chlamydia pneumoniae*-induced atherosclerosis. *J Infect Dis* 2000;181(3):S514–18.
- 81 Handin R: Disorders of coagulation and thrombosis (chapter 118); in Fauci A, et al (eds): *Principles of Internal Medicine*. New York, McGraw-Hill, 1998, pp 736–743.
- 82 Cosgriff TM: Mechanisms of disease in Hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. *Rev Infect Dis* 1991;13(1):97–107.
- 83 Petkova SB, Huang H, Factor SM, Pestell RG, Bouzahzah B, Jelicks LA, Weiss LM, Douglas SA, Wittner M, Tanowitz HB: The role of endothelin in the pathogenesis of Chagas' disease. *Int J Parasitol* 2001;31(5–6):498–510.
- 84 NIH-NHLBI: www.nhlbi.nih.gov/health/public/heart/other/hrt_lung.htm.
- 85 NIH-NHLBI: www.nhlbi.nih.gov/health/public/heart/other/chdfacts.htm.
- 86 Sykes M: Immunobiology of transplantation. *Faseb J* 1996;10(7):721–730.
- 87 Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, Croker BP, Demetris AJ, Drachenberg CB, Fogo AB, Furness P, Gaber LW, Gibson IW, Glotz D, Goldberg JC, Grande J, Halloran PF, Hansen HE, Hartley B, Hayry PJ, Hill CM, Hoffman EO, Hunsicker LG, Lindblad AS, Yamaguchi Y, et al: The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999;55(2):713–723.
- 88 Ross R: Atherosclerosis: a question of endothelial integrity and growth control of smooth muscle. *Harvey Lectures* 1982;77:161–182.
- 89 Hruban R, Beschoner W, Baumgartner W, Augustine S, Ren H, Reitz B, Hutchins G: Accelerated arteriosclerosis in heart transplant recipients is associated with a T-lymphocyte-mediated endothelialitis. *Am J Pathol* 1990;137:871–882.
- 90 De Waal RM, Bogman MJ, Maass CN, Cornelissen LM, Tax WJ, Koene RA: Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts. *Nature* 1983;303(5916):426–429.
- 91 Shi C, Lee WS, He Q, Zhang D, Fletcher D Jr, Newell J, Haber E: Immunologic basis of transplant-associated arteriosclerosis. *Proc Natl Acad Sci U S A* 1996;93:4051–4056.
- 92 Glas R, Franksson L, Öhlen C, Höglund P, Koller B, Ljunggren HG, Kärre K: Major histocompatibility complex I-specific and -restricted killing of β2-microglobulin-deficient cells by CD8⁺ cytotoxic T lymphocytes. *Proc Natl Acad Sci USA* 1992;89:11381–11385.
- 93 Zijlstra M, Auchincloss H Jr, Loring J, Chase C, Russell P, Jaenish R: Skin graft rejection by β2-microglobulin-deficient mice. *J Exp Med* 1992;175:885–893.
- 94 Quinn DG, Zajac AJ, Hioe CE, Frelinger JA: Virus-specific, CD8⁺ major histocompatibility complex class I-restricted cytotoxic T lymphocytes in lymphocytic choriomeningitis virus-infected beta2-microglobulin-deficient mice. *J Virol* 1997;71(11):8392–8396.
- 95 Shi C, Feinberg MW, Zhang D, Patel A, Sim CU, Dong ZM, Chapman SM, Gutierrez-Ramos JC, Wagner DD, Sibinga NE, Haber E: Donor MHC and adhesion molecules in transplant arteriosclerosis. *J Clin Invest* 1999;103(4):469–474.
- 96 Maier S, Tertilt C, Chambron N, Gerauer K, Huser N, Heidecke CD, Pfeffer K: Inhibition of natural killer cells results in acceptance of cardiac allografts in CD28^{-/-} mice. *Nat Med* 2001;7(5):557–562.

- 97 Hughes CC, Pober JS: Costimulation of peripheral blood T cell activation by human endothelial cells. Enhanced IL-2 transcription correlates with increased c-fos synthesis and increased Fos content of AP-1. *J Immunol* 1993;150(8 Pt 1):3148–3160.
- 98 St. Louis J, Lederer J, Lichtman A: Costimulator deficient antigen presentation by an endothelial cell line induces a nonproliferative T cell activation response without anergy. *J Exp Med* 1993; 178:1597–1605.
- 99 Kosaka H, Surh CD, Sprent J: Stimulation of mature unprimed CD8+ T cells by semiprofessional antigen-presenting cells in vivo. *J Exp Med* 1992; 176(5):1291–1302.
- 100 Geppert T, Lipsky P: Antigen presentation by interferon-gamma-treated endothelial cells and fibroblasts: differential ability to function as antigen-presenting cells despite comparable Ia expression. *J Immunol* 1985;135:3750–3762.
- 101 Vora M, Yssel H, de Vries J, Karasek M: Antigen presentation by human dermal microvascular endothelial cells. Immunoregulatory effect of IFN- γ and IL-10. *J Immunol* 1994;152:5734–5741.
- 102 Briscoe DM, Henault LE, Geehan C, Alexander SI, Lichtman AH: Human endothelial cell costimulation of T cell IFN-gamma production. *J Immunol* 1997;159(7):3247–3256.
- 103 Hughes C, Savage C, Pober JS: Endothelial cells augment T cell interleukin 2 production by a contact-dependent mechanism involving CD2/LFA-3 interaction. *J Exp Med* 1990;171(1453):1453–1467.
- 104 Savage C, Hughes C, Pepinsky R, Wallner B, Freedman A, Pober J: Endothelial cell Lymphocyte Function-associated Antigen-3 and an unidentified ligand act in concert to provide costimulation to human peripheral blood CD4+ T cells. *Cell Immunol* 1991;137:150–163.
- 105 Perez VL, Henault L, Lichtman AH: Endothelial antigen presentation: stimulation of previously activated but not naive TCR-transgenic mouse T cells. *Cell Immunol* 1998;189(1):31–40.
- 106 Ma W, Pober JS: Human endothelial cells effectively costimulate cytokine production by, but not differentiation of, naive CD4+ T cells. *J Immunol* 1998;161(5):2158–2167.
- 107 Epperson D, Pober J: Antigen-presenting function of human endothelial cells. Direct activation of resting CD8 T cells. *J Immunol* 1994;153:5402–5412.
- 108 Dengler TJ, Johnson DR, Pober JS: human vascular endothelial cells stimulate a lower frequency of alloreactive CD8(+) Pre-CTL and induce less clonal expansion than matching B lymphoblastoid cells: development of a novel limiting dilution analysis method based on CFSE labeling of lymphocytes. *J Immunol* 2001;166(6):3846–3854.
- 109 Biedermann BC, Pober JS: Human vascular endothelial cells favor clonal expansion of unusual alloreactive CTL. *J Immunol* 1999;162(12):7022–7030.
- 110 Hancock WW, Sayegh MH, Zheng XG, Peach R, Linsley PS, Turka LA: Costimulatory function and expression of CD40 ligand, CD80, and CD86 in vascularized murine cardiac allograft rejection. *Proc Natl Acad Sci USA* 1996;93(24):13967–13972.
- 111 Ehrlich H: Culture of Aorta; in Harris C, Trump B, Stoner G (eds): *Methods in Cell Biology, Normal Human Tissue and Cell Culture A* (Respiratory, Cardiovascular, and Integumentary Systems). New York, Academic Press, 1980, pp 117–134.
- 112 Striker G, Harlan J, Schwartz S: Human endothelial cells in vitro; in Harris C, Trump B, Stoner G (eds): *Methods in Cell Biology, Normal Human Tissue and Cell Culture A* (Respiratory, Cardiovascular, and Integumentary Systems). New York, Academic Press, 1980, pp 136–150.
- 113 Roskelley CD, Desprez PY, Bissell MJ: Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction. *Proc Natl Acad Sci U S A* 1994;91(26):12378–12382.
- 114 Theopold U, Kohler G: Partial tolerance in beta-galactosidase-transgenic mice. *Eur J Immunol* 1990;20(6):1311–1316.
- 115 Borrow P, Cornell JL, Ruppe MD, Mucke L: Immunization-induced inflammatory infiltration of the central nervous system in transgenic mice expressing a microbial antigen in astrocytes. *J Neuroimmunol* 1995;61(2):133–149.
- 116 Aird W, Edelberg J, Weiler-Guetter H, Simmons W, Smith T, Rosenberg R: Vascular bed-specific expression of an endothelial cell gene is programmed by the tissue microenvironment. *J Cell Biol* 1997;138:1117–1124.
- 117 Schlaeger TM, Bartunkova S, Lawitts JA, Teichmann G, Risau W, Deutsch U, Sato TN: Uniform vascular-endothelial-cell-specific gene expression in both embryonic and adult transgenic mice. *Proc Natl Acad Sci U S A* 1997;94(7):3058–3063.
- 118 Jax: jaxmice.jax.org/library/notes/447c.html.
- 119 Price J, Turner D, Cepko C: Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. *Proc Natl Acad Sci USA* 1987; 84(1):156–160.
- 120 Williams RS, Johnston SA, Riedy M, DeVit MJ, McElligott SG, Sanford JC: Introduction of foreign genes into tissues of living mice by DNA-coated microprojectiles. *Proc Natl Acad Sci USA* 1991;88(7):2726–2730.
- 121 Tang DC, DeVit M, Johnston SA: Genetic immunization is a simple method for eliciting an immune response. *Nature* 1992;356(6365):152–154.
- 122 Corr M, Lee DJ, Carson DA, Tighe H: Gene vaccination with naked plasmid DNA: mechanism of CTL priming. *J Exp Med* 1996;184(4):1555–1560.
- 123 Leclerc C, Deriaud E, Rojas M, Whalen R: The preferential induction of a Th1 immune response by DNA-based immunization is mediated by the immunostimulatory effect of plasmid DNA. *Cell Immunol* 1997;179:97–106.
- 124 Pfeifer JD, Wick MJ, Roberts RL, Findlay K, Normark SJ, Harding CV: Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature* 1993;361(6410):359–362.
- 125 Fenton RG, Keller CJ, Hanna N, Taub DD: Induction of T-cell immunity against Ras oncoproteins by soluble protein or Ras-expressing *Escherichia coli*. *J Natl Cancer Inst* 1995;87(24):1853–1861.
- 126 Speidel K, Osen W, Faath S, Hilgert I, Obst R, Braspenning J, Momburg F, Hammerling G, Ramnensee H: Priming of cytotoxic T lymphocytes by five heat-aggregated antigens in vivo: conditions, efficiency, and relation to antibody responses. *Eur J Immunol* 1997;27:2391–2399.
- 127 Johnson D, Faherty D, Zauderer M: TTGG-A – L-specific memory B cells induced in low responder strains. *J Immunol* 1984;137:2791–2795.
- 128 Brubaker JO, Thompson CM, Morrison LA, Knipe DM, Siber GR, Finberg RW: Th1-associated immune responses to beta-galactosidase expressed by a replication-defective herpes simplex virus. *J Immunol* 1996;157(4):1598–1604.
- 129 Kyburz D, Aichele P, Speiser DE, Hengartner H, Zinkernagel RM, Pircher H: T cell immunity after a viral infection versus T cell tolerance induced by soluble viral peptides. *Eur J Immunol* 1993;23(8): 1956–1962.
- 130 Newman K, Dunn P, Owens J, Schulick A, Virmani R, Sukhova G, Libby P, Dichek D: Adenovirus-mediated gene transfer into normal rabbit arteries results in prolonged vascular cell activation, inflammation, and neointimal hyperplasia. *J Clin Invest* 1995;96:2955–2965.