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Human T-Cell Development and Thymic Egress: An Infectious Disease Perspective

Rachel S. Resop^{a,b,*} and Christel H. Uittenbogaart^{a,b,c,d,*}

^aDepartment of Microbiology, Immunology, and Molecular Genetics, David Geffen Medical School at UCLA, Los Angeles, CA 90095

^bDepartment of Pediatrics, David Geffen Medical School at UCLA, Los Angeles, CA 90095

^cUniversity of California at Los Angeles AIDS Institute, Los Angeles, CA 90095

^dJonsson Comprehensive Cancer Center, David Geffen Medical School at UCLA, Los Angeles, CA 90095

Abstract

Emigration of mature naïve CD4 SP T cells from the human thymus to the periphery is not fully understood, although elucidation of the mechanisms that govern egress of T cells is crucial to understanding both basic immunology and the immune response in diseases such as HIV infection. Recent work has brought to light the requirement for sphingosine-1-phosphate (S1P) and its receptors in a variety of fields including mature naïve T-cell egress from the thymus of mice. We are examining the expression and function of this novel requisite T-cell egress receptor within the human thymus, characterizing changes observed in the expression and function of this receptor in infectious diseases. To perform this work, we use a variety of humanized murine models reviewed in this article. Future work in the field of T-cell egress, especially as it pertains to S1P receptors, should advance the fields of basic T-cell immunology and immunopathology and open new avenues for exploration into novel therapeutics.

Keywords

human immunodeficiency virus; human immunology; humanized mice; migration; murine models; S1P-R1; S1P-R2; sphingosine-1-phosphate; T-cell reconstitution; thymic egress; thymocyte development; thymus

I. HISTORY AND BASICS OF T-CELL IMMUNOLOGY

The field of immunology is relatively young compared to other biological sciences. Edward Jenner, an English physician-scientist and discoverer of the concept of vaccination, which continues to be used today, is credited with stimulating the origination of the field and is often referred to as one of the “fathers of immunology,” along with Paul Ehrlich and Louis Pasteur. Although the theory of vaccination had circulated for some time before his work,

*Address all correspondence to: Christel H. Uittenbogaart, M.D. or Rachel S. Resop, Ph.D., 615 Charles E. Young Drive South, BSRB 173, Mail code 736322, Los Angeles, CA 90095. uittenbo@ucla.edu or resopr@g.ucla.edu.

Jenner cemented the validity of the notion when he intrepidly attempted to protect individuals from deadly smallpox infection by using a closely related nonfatal infectious agent, cowpox, that he administered to healthy individuals in 1796. Jenner documented that treatment with cowpox resulted in immunity to smallpox. Although he was successful, it would be some time before the underlying mechanisms of his discovery were fully understood.¹ This pattern of an observation followed by toils to uncover a mechanism would set the tone for the field, in which researchers continue to strive to understand the processes governing the human immune system and the mechanisms of our incredibly intricate and complex response to pathogens that threaten to perturb the delicate balance of a healthy state.

Our current understanding is that our immune system is comprised of two branches known as innate and adaptive immunity. Innate immunity refers to immune system cells that are always ready to respond to a wide range of pathogens. This response is nonspecific and involves cells such as granulocytes, NK cells, and macrophages (phagocytic cells capable of engulfing many potentially harmful microorganisms).² Elie Metchnikoff, a Russian immunologist, is responsible for primarily characterizing these types of reactions.^{1,3} Adaptive immunity refers to a more specific response that the immune system generates over time. Immunologic memory is developed upon encounter with a particular antigen (harmful microorganism or other substance), such that if the antigen presents again, specific cells are primed to deal with it. Adaptive immunity is further divided into two branches: humoral and cellular.

B lymphocytes (B cells, from “bursa” of the chicken, where they were discovered), responsible for humoral immunity, generate antibodies that recognize antigens and can neutralize recurring pathogens. T lymphocytes or T cells (from thymus, where these cells complete their development) are key players in adaptive immunity, specifically cell-mediated immunity. Briefly, T cells are comprised of several subsets, each of which is responsible for a specific task in the immune response. These tasks include cytotoxic activity (direct cell killing by Cluster of Differentiation 8⁺ [CD8⁺] T cells or cytotoxic T lymphocytes [CTLs]), regulation of other immune cells (CD4 and CD8 regulatory T cells [CD4/CD8 Tregs]), helper activity for CD4 T lymphocytes (Th1, Th2, and T follicular helper [Tfh] cells), and protective immunity against extracellular bacteria and fungi (Th1, Th2, Th17, Th22, Tfh, and $\gamma\delta$ T cells). Development of these various subsets begins in the thymus, but differentiation into the specific subsets described above occurs in the periphery after egress from the thymus.¹ T-cell development in the thymus is discussed below.

II. T-CELL DEVELOPMENT IN THE THYMUS AND T-CELL SELECTION

As discussed in the preceding section, the adaptive branch of the human immune system is quite specific and potent. Therefore, the participants in adaptive immunity must be trained to avoid self-reactivity and to identify and target foreign invaders with an adequately strong response. T and B lymphocytes undergo intense selection processes during development. In this section, we focus on T cells, although it bears mentioning that B cells undergo an analogous process of selection and maturation within the bone marrow^{4,5} and the germinal centers of the secondary lymphoid organs (spleen and lymph nodes).^{6–8}

T cells ultimately develop from CD34⁺ hematopoietic stem-cell progenitors that originate in bone marrow.⁹ In fact, both T- and B-cell precursors originate within bone marrow, but whereas B cells continue their development therein, T-cell precursors emigrate to the thymus, a lymphoid organ located in the thoracic cavity over the heart, where they progress through several stages of development.¹ Upon entry of these precursors into the thymus, they have not yet undergone rearrangement of the genes encoding the T cell receptor (TCR) (a heterodimer consisting of an α and β chain embedded in a CD3 complex that allows recognition of the major histocompatibility complex [MHC]-bound antigen presented to T cells, the expression of which is a defining characteristic of T cells),¹⁰ a process that occurs during their maturation in the thymus. Additionally, at this stage they lack most of the surface molecules used to characterize mature T cells. Various surface-expressed receptors and other molecules are used to characterize the maturation stages of T cells, because in varied combinations they are quite specific to stages of T-cell development in the thymus. T-cell precursors in the thymus undergo extensive proliferation, but the majority of these cells die and do not progress along the differentiation pathway to become mature T cells.¹¹

Within the thymic cortex, or the outer portion of its lobes, initial differentiation toward the T-cell pathway occurs, encouraged by the interaction between T-cell precursors and thymic stromal cells. The TCR gene begins to rearrange, and CD4, a coreceptor for the TCR, is expressed on thymocytes. T cells are considered double-positive cells once CD8 is also up-regulated. The first selection process, termed positive selection, selects for CD4⁺/CD8⁺ thymocytes that recognize the MHC displayed via specialized antigen-presenting cells in the thymus.^{12,13} Next, thymocytes up-regulate CCR7, which is necessary for their translocation to the thymic medulla,^{14,15} guided by additional cytokines produced by stromal cells.¹⁶ Once in the medulla, the thymocytes undergo a second selection process, negative selection, whereby double-positive cells that react too strongly to self-MHC fail to receive survival signals^{17,18} and undergo programmed cell death, or apoptosis,¹⁹ whereas cells that react mildly are selected for survival.¹⁸ The selection process is not yet fully understood, but recent evidence suggests that in developing thymocytes, microRNAs have a role in selection.²⁰ Upon loss of expression of CD4 or CD8, thymocytes are considered single-positive (SP) cells.

Typical developmental markers for thymocytes include CD27 (tumor necrosis factor [TNF] receptor), CD69 (C-type lectin transmembrane protein), CD45RA (leukocyte common antigen), and CD62 ligand (CD62L, also known as L selectin). CD27 is also expressed on all thymic medullary cells and is used as a marker of relatively mature human thymocytes.^{21,22} CD45 is found in two isoforms, CD45RA and CD45RO, that are expressed differentially on various developing thymocyte subsets. CD45RA is expressed during the most immature stage of thymocyte development, not expressed in intermediate stages, and finally reexpressed in mature thymocytes. CD45RO is expressed on the majority of CD4⁺CD8⁺ (double-positive), intermediate-stage thymocytes.²³ CD69, an activation marker that begins to be expressed during positive selection² and is also expressed on activated peripheral T cells, has recently been found to have a role in regulation of T-cell egress.² CD69 is down-regulated on mature thymocytes before egress and is not present on recent thymic emigrants.²⁴ Finally, thymocytes up-regulate CD62L and are able to egress the thymus for the periphery to encounter their antigens as mature SP T cells. Egress from the thymus is

discussed in the following section. See Figure 1 for the markers used to identify maturing thymocyte populations.

During thymocyte maturation, transient expression of KLF2, a master transcriptional regulator, has been shown in the mouse to control the expression of several migration-associated surface molecules expressed on thymocytes, including CD62L and CCR5.^{26–28} KLF2 begins to be expressed on mature thymocytes nearly prepared to egress the thymus for the periphery and has been demonstrated to regulate transcription of sphingosine-1-phosphate receptor 1 mRNA (S1P-R1, an egress-associated receptor discussed in detail below) in murine models.²⁶

III. MATURE THYMOCYTE EGRESS FROM THE THYMUS

After completing their development in the thymus, thymocytes that are prepared to egress the thymus for the periphery are considered mature naïve T lymphocytes. Our work and that of other investigators has determined the phenotype of these cells to be CD3hiCD27⁺CD45RA⁺CD62L⁺CD69[–].^{29–31} As shown by Vanhecke et al., CD69 is lost before thymocyte egress of mature naïve cells²⁴ and can therefore be used reliably as a marker of fully mature thymocytes prepared for egress, in conjunction with the additional surface markers above. Interestingly, these mature T cells are refractory to death by apoptosis (programmed cell death), in contrast to less mature subsets, which are susceptible to apoptosis. Instead, mature SP cells respond to TCR stimulation by proliferating.³² They also must be able to respond to signals that “tell” them it is time to egress the thymus for the periphery. Once they leave the thymus, these cells are known as recent thymic emigrants (RTEs; Figure 2).

Many of the chemokine receptors and chemokines involved in egress from the thymus have been well characterized, but this work has been performed predominantly in murine models and may not necessarily reflect the situation in humans. Therefore, it is important to consider that the current understanding of the process of thymic egress in humans is quite fluid and thus subject to fine-tuning as methods emerge to permit in-depth work in humans or as more researchers begin to work with human tissue samples. Briefly, it is known that the chemokine receptor CXCR4 and its ligand CXCL12 are important molecules involved in egress from the thymus to the periphery. CXCL12, also known as stromal-derived factor 1 (SDF-1), is a potent lymphocyte chemoattractant known to retain thymocytes at the corticomedullary junction,³³ the region of the thymus microenvironment at which thymocytes enter and exit the thymus until the cells are “told” to leave.³⁴ Additional factors such as the early growth response gene (*Egr1*, a transcriptional regulator involved in differentiation and mitogenesis),³⁵ integrin $\alpha 5\beta 1$ (a mediator of migration and proliferation),^{36,37} aryl hydrocarbon receptor (AHR, a helix-loop-helix transcription factor),^{38,39} laminin-5 (a mediator of migration, organization, and attachment in tissues),⁴⁰ CCR7 (a mediator of migration from the cortex to the medulla during selection),^{14,41,42} KLF2 (see above),²⁷ PI3 kinase (PI3K, a negative regulator of KLF2),⁴³ and phosphatase and tensin homolog (PTEN, a regulator of PI3K)⁴⁴ also bear mentioning because they have been shown to have roles in the process of egress (although much of the research is murine work). According to “textbook knowledge,” this maturation process takes ~2 weeks.⁴⁵

However, it has recently been discovered that the time between positive selection and emigration is much less than previously believed: 4 or 5 days as opposed to the earlier estimate of 14 days. Additionally, emigration may not be stochastic as previously believed, but, rather, some data point toward the most immature thymocytes leaving before less mature medullary cells, although this has not been unequivocally demonstrated.^{32,46,47}

There are limited data on the mechanisms regulating egress of mature human thymocytes from the thymus to the periphery. Murine studies investigated the S1P/S1P-R1 ligation prerequisite for thymocyte egress and demonstrated that S1P-R1 is essential for thymic egress of mature SP murine thymocytes.^{48,49} However, no reports to date have demonstrated whether the S1P/S1P receptor pathway has a role in the response of mature thymocytes to S1P or egress from the human thymus to the peripheral blood and lymphoid tissues; moreover, the exact population of thymocytes that expresses S1P-R1 has not been fully characterized in either mice or humans.

The following section describes the role of sphingosine-1-phosphate in the egress of mature, SP CD4 and CD8 thymocytes from the thymus to the periphery in mice and humans, the characterization of which is the main focus of this review.

IV. SPHINGOSINE-1-PHOSPHATE AND ITS ROLE IN CHEMOTAXIS

S1P is a signaling sphingolipid molecule, also known as a lysosphingolipid, with multiple tasks throughout the body.⁵⁰ The name “sphingosine” in fact originates from the mythological Greek character the sphinx, in reference to the molecule’s promiscuous nature in the human body.⁵¹ S1P functions in a myriad of roles in humans, from regulation of cell death (specifically, suppression of apoptosis)⁵² and survival,^{53,54} immune responses,⁵⁵ autoimmune conditions and allergies,^{56–58} B-cell development,⁵⁹ insulin modulation,⁶⁰ cell motility,^{61,62} and response to viral infections.^{63,64} In addition, S1P is an “integral constituent” of high-density lipoprotein (HDL) complexes in the plasma and not only plays a role in regulation of its bioactivity but also contributes to protection against atherosclerosis.^{65–67} The S1P molecule is comprised of a long saturated carbohydrate chain and one alcohol, amino, and phosphate group.⁶⁸ S1P is produced by phosphorylation of its precursor, sphingosine, by sphingosine kinase, which is found ubiquitously in the cytoplasm and endoplasmic reticulum of many cell types^{69,70} and can also be released from ceramides via their conversion to sphingosine and subsequent phosphorylation.⁷¹

Among its many roles, S1P is crucial for proper lymphocyte trafficking between the lymphoid organs and about the periphery^{48,72} and the trafficking of various other cell types such as smooth muscle and endothelial cells.⁷³ An S1P gradient is present in which S1P is concentrated in the blood (about 100 nM) where it is taken up, phosphorylated, stored, transported, and released by erythrocytes^{74,75} and activated platelets⁷⁶ in response to factors in the serum⁷⁵ but is at a low concentration in lymphoid tissues. S1P is degraded by sphingosine lyase in lymphoid tissues, thus keeping tissue concentrations low⁷⁷; sphingosine phosphatase also aids in breaking down S1P in tissues.⁷⁸ Sphingosine kinase, sphingosine phosphatase, and sphingosine lyase together maintain the S1P gradient (see also Figure 3), which promotes the influx of lymphocytes bearing one of its five G-protein-coupled

receptors to the lymph nodes and other lymphoid tissues. The S1P receptor family is known to include S1P-R1-5; these receptors are present on various cell types.⁷⁹ S1P receptors had not been characterized on human thymocytes before our recent work (Resop et al. *Journal of Allergy and Clinical Immunology*. In press, DOI 10.1016/j.jaci.2015.12.1339), although murine work alluded to their importance in emigration from the thymus.⁴⁸

In murine models, it has been shown that S1P/S1P-R1 ligation is required for mature T cells to egress the thymus to the periphery. Mice with siRNA-ablated S1P-R1 have virtually no naïve CD4 or CD8 SP cells in the peripheral lymph nodes; treatment of mice with FTY720, an immune modulator that targets S1P-R1, 3, 4, and 5, recapitulates this effect.^{48,49} S1P-R1 also has a role in migration of T cells to and from peripheral lymphoid organs.⁸⁰ S1P-R2 has been shown to inhibit migration of maturing B cells in the germinal centers when ligated to S1P.⁸¹ However, no studies have demonstrated the role of S1P and its receptors in human thymocyte egress.

V. ADDITIONAL MARKERS FOR MATURATION STAGES OF THYMOCYTES IN THE HUMAN THYMUS

As described above, various markers are used to identify the stages of thymocyte development, including CD3, CD4, CD8, CD45RA, CD27, CD69, and CD62L. CD31, also known as PECAM-1, is well known as a marker of RTEs and interestingly as a marker of CD4⁺ (but not CD8⁺) RTE in HIV patients,⁸² although additionally it has been reported that CD31 is expressed on human thymocytes.⁸³ CD31 has been shown to have the ability to modulate the TCR activation threshold via activity of tyrosine phosphatases and may therefore have a role in the actual T-cell selection process.^{84,85} Additionally, the characterization of CD31 expression on thymocytes is ongoing and applies to the field of thymocyte egress and the characterization of receptors and ligands involved in human that CD31 may additionally be expressed in mature thymocytes prepared for egress (and, thus, its expression may correlate with that of other factors responsible for egress). Perhaps, CD31 may function synergistically with other chemotaxis receptors to allow egress of mature thymocytes from thymus to periphery; it will be intriguing to follow the emergence of new data in this field in the coming years.

VI. MURINE MODELS AND THEIR RELEVANCE FOR HUMAN IMMUNOLOGY

The adaption of mouse models to the study of mechanisms of immune function was a scientific milestone and a key step in achieving a greater understanding of the human immune system. According to H.C. Morse, a researcher who was at the forefront of the development of murine models for research, “Our current realization of the potential of the inbred mouse has developed over a period of more than 70 years and reflects the dedicated research of many skilled and imaginative scientists.”⁸⁶ The majority of the facts discussed above, including the characterization of T-cell development in the thymus, the various facets of the lymphocyte repertoire, and the necessity for cytokines such as S1P for T-cell egress from the thymus, were made possible by the use of mice in research.^{45,48,87,88} Murine models began to be used in 1929 with the establishment of the Jackson Laboratory and the development of the first inbred mouse strain,^{89,90} which eventually led to the development

of various models of inbred mice designed to gain a better understanding of human diseases.^{86,91} With a repertoire of model systems in which we may manipulate various aspects, such as, for example, knocking out the expression of a specific gene, researchers are able to observe the resultant phenotype and thus infer the function of a particular facet of the immune system. Additionally, murine models of a wide range of diseases and autoimmune conditions have been developed. Moreover, mice closely resemble humans in regard to many aspects of the immune system (however, there are indeed many differences, described below), are relatively simple to work with, and have a short reproductive time frame; thus, they serve as invaluable modern investigative tools.

Murine work is an adequate model of the immune system of humans in many ways. Like humans, mice develop T, B, NK and dendritic cells; macrophages; monocytes; and other important immune cells. Many of the human cytokines studied in immunology, or analogous cytokines, are also produced in mice, although the effects of cytokines can vary.^{92,93} Recent reports indicate that only ~300 genes differ between mice and humans.⁹⁴ However, there exist several discrepancies between the mouse and human immune system, evidenced in both adaptive and innate branches of the immune system.⁹⁵ These differences may influence the interpretation of results of immunological research, in the aim of understanding the basics of immune system development, activation, and response to challenge. Some of the key areas in which differences are observed between mouse and human include balance of lymphocyte subsets; B- and T-cell signaling pathways; T-helper 1 and 2 (Th1 and Th2) cells; certain cytokines, chemokines, and their receptors; toll-like receptors; antigen presentation by endothelial cells; and expression and function of costimulatory molecules.⁹⁶ A recent report also demonstrated that genes that are up- or down-regulated in response to various immunological challenges (i.e., infectious agents/exotoxins, burn trauma, etc.) differ significantly in mice and humans; the authors concluded that genomic responses in mice “poorly mimic response in humans.”⁹⁷

In an attempt to address the above issues, humanized mouse models have been developed. Notably, the majority of humanized mouse models evolved from HIV research laboratories, because researchers needed a way to examine the effect of HIV on humans and found traditional murine systems inadequate. The severe combined immunodeficient (SCID) mouse was the first such model to be used.^{98,99} Today, several more advanced humanized mouse models exist, and the underlying shared feature of all models is a reconstitution in the mouse of a “human-like” immune system. In general, genetic manipulation renders certain aspects of the murine immune system ineffective; components such as murine T, B, and NK cells do not develop, and human lymphocyte precursors are normally introduced that develop into human immune cells in the mouse.¹⁰⁰ The immunodeficient nonobese diabetic (NOD), severe combined immunodeficient, common γ -chain knockout ($\gamma c^{-/-}$) (NSG) thy/liv model is generated by (1) a NOD genetic background that reduces innate immunity in the mouse, resulting in macrophages and dendritic cells that are not functional, (2) a SCID mutation (loss-of-function mutation in the *Prkdc* gene, which is responsible for repairing the double-stranded DNA break that occurs during v-d-j recombination in T- and B-cell development), resulting in compromised adaptive immunity (does not develop) in T and B cells, and (3) a common γ -chain mutation (the *IL2ry* gene) that compromises a common key component of the receptor for six interleukins (IL-2, 4, 7, 9, 15, and 21), resulting in

murine NK cells that do not develop. These mice may be implanted with human fetal thymus and liver tissue and injected with human fetal CD34⁺ hematopoietic progenitor cells, which develop in the thymus into functional “human-like” T lymphocytes (this type of mouse is commonly known as a BLT mouse.) B and NK cells of human origin also develop in the animal, allowing a strikingly accurate reconstruction of a humanoid immune system within the mouse.^{101,102} For a view of the generation of the NSG thy/liv mouse, see Figure 4.

Thus, the NSG thy/liv mouse, as well as other humanized mouse models, have come into favor for modeling HIV infection and are beginning to be used to model other human diseases as well, because they appear to more accurately represent the human immune response to challenge. For example, in HIV infection of the BLT mouse, the cellular response observed, as well as the ability of the virus to escape the immune response mounted by the host, is similar to that observed in humans.^{103–105} It is crucial to continue to expand the use of humanized mouse models to research of a wide variety of disease states, considering that several therapies that have been demonstrated to work in mouse models have failed to have the desired effect in human trials.^{106–111} Additionally, multiple differences exist between murine and human thymus immunology, which must be taken into consideration when using animal models to investigate human thymocyte development and egress. The human response to HIV infection, particularly in the thymus, is discussed below.

VII. HIV INFECTION AND INFECTION OF THE THYMUS

A. Human Immunodeficiency Virus

The prelude to what became known as acquired immunodeficiency syndrome (AIDS) was first described in the early 1980s as a mysterious cluster of symptoms, including Kaposi's sarcoma, that appeared to disproportionately target homosexual men, injection drug users, hemophiliacs, and Haitians.^{112–118} The disease remained an enigma during the early 1980s and caused considerable fear among the public and controversy over extremist sentiments regarding the groups of individuals affected.^{119,120} The infectious agent responsible for AIDS, a virus with similar characteristics to known lentiviruses, was reported simultaneously in 1983 by French and American research groups led by Luc Montagnier and Robert Gallo,^{121,122} who would later vie for credit for the discovery. Following its initial characterization, virologists and immunologists raced to learn more about the infectious agent. It was soon discovered that the HIV targets lymphocytes, or cells of the immune system, especially T cells, within which it replicates,¹²³ and that the virus uses CD4¹²⁴ as well as one of two coreceptors, CXCR4 or CCR5¹²⁵ for infection of T cells. Further characterization of the virus itself revealed it to be a retrovirus, more specifically, a lentivirus (a subset of retroviruses) comprised of two copies of single-stranded RNA capable of reverse transcription (creation of DNA from RNA) and integration into the host genome.¹²⁵ The virus wreaks havoc on the immune system in many ways, but most significantly by depleting CD4⁺ T cells, which impairs cell-mediated immunity and eventually allows opportunistic infections that the host would normally be able to clear to cause irreversible damage.^{126–128}

Transmission of HIV most commonly occurs via sexual contact, resulting in exposure to either cell-associated or free infectious virus particles.¹²⁹ This route of infection includes

male-to-female, female-to-male, or male-to-male sexual transmission across mucosal (epithelial cell) surfaces.¹³⁰ Less commonly, HIV is transmitted via intravenous drug use or the sharing of needles for other purposes,¹³¹ intrauterine or mother-to-child transmission during birth,¹³² or contact with infected bodily fluids including blood,¹³³ semen,¹³⁴ breast milk,^{135,136} or vaginal fluid.¹³⁷ The basic mechanisms of HIV infection, epidemiology of the disease, and the immune response to infection are reviewed in several manuscripts;^{129,138–144} the subject of this review is the potential effect of established HIV-1 infection on the specific requisite thymocyte egress-mediating cell-surface receptor S1P-R1.

Perturbations in lymphocyte count, cell populations, and cytokines during HIV-1 infection all have the potential to affect expression of cell-surface receptors. This has not been examined as it pertains to thymocyte egress receptors but has been considered in other systems.^{145,146} Cytokine perturbations during HIV infection have mostly been characterized in the periphery and not within the thymus proper. For example, it has been well documented that many proinflammatory cytokines peak several days after an initial infection event, and each cytokine has its own corresponding time point postinfection at which it peaks in concentration in the periphery.¹⁴⁷

B. Typical Infection of the Human Thymus

HIV infection spreads to various organs of the body, including the thymus.^{148–151} CD4 is expressed in a large proportion of thymocytes including double-positive and single (CD4)-positive cells, CXCR4 is expressed at high levels within the cortical subset (mainly double-positive cells), and CCR5 is expressed within the medullary subset (mainly mature SP cells). Thus, there are ample targets for both CXCR4 and CCR5-tropic HIV infection within the thymus.¹⁵² Interestingly, we have observed that CD25⁺FoxP3⁺ thymic Tregs are preferentially infected by CCR5-tropic HIV-1, whereas CXCR4-tropic virus does not readily infect this thymic subset (unpublished data).¹⁵³ During the course of infection, susceptible subsets are depleted (Resop R, unpubl.),^{148,154} leaving immature double-negative thymocytes and some SP cells (especially CD8 SP cells) remaining. Moreover, CD4 is down-regulated on infected T cells by gp120, an HIV accessory protein^{155,156} contributing not only to a depleted thymus but also a significantly altered profile of the remaining cells therein and a skewed peripheral T-cell profile as well.¹⁵⁷ However, the thymus continues to contribute to reconstitution. The extent to which it may perform this duty depends on the success of highly active antiretroviral therapy (HAART) treatment within the individual, the stage of HIV infection, and the age of the infected individual, among other factors.¹⁵⁴

Because these important changes in the human thymus during HIV infection needed to be examined, a model system was necessary. Fortunately, humanized mice are well suited for the modeling of HIV infection of the thymus. In SCID and recombinant activating gene (RAG^{-/-}) thy/liv mice infected with CCR5 (R5)-tropic or CXCR4 (X4)-tropic HIV-1 (the two tropisms of HIV, using the CCR5 or CXCR4 coreceptor) intraperitoneally or intrathymically, the thymus becomes robustly infected after several weeks.¹⁵⁸ We have observed that cytokines, including interferon- α (IFN- α) and TNF- α are elevated in intrathymically infected human fetal thymic implants in immunodeficient mice by 5 weeks postinfection (Resop et al., manuscript in preparation). Intriguingly, a transient boost in

thymopoiesis following an initial infection event has been observed in models of simian immunodeficiency virus (SIV),^{159–161} but the mechanisms involved or the duration of this effect of infection have not yet been elucidated.

VIII. CONCLUDING REMARKS

The role of S1P and its receptors plays a part in the egress of mature human thymocytes from the thymus to the periphery, and the only work on this subject to date had been performed in mice. Seminal work by Matloubian et al., showing that S1P1^{−/−} hematopoietic cells remained sequestered in the thymus after development into mature CD4⁺ and CD8⁺ T cells and could not egress to the periphery, was performed in a traditional (nonhumanized) mouse model that could not be assumed to reflect the function of the S1P/S1P receptor system in humans.⁴⁸ Thus, it is imperative to examine the function of the S1P/S1P receptor system in the human thymus and to identify and characterize the populations expressing S1P receptors and those that respond to S1P.

Because HIV infection results in extensive T-cell depletion, there is an urgent need to examine approaches for reconstitution of the T-cell “repertoire” in HIV-infected individuals. Reconstitution of a peripheral repertoire depends largely on functional and enhanced thymopoiesis or generation of mature naïve T cells in the thymus. The egress of mature T cells following development is critical to reconstitution and had thus far only been examined superficially; Dion et al. and other investigators noted a transient increase in T-cell output in the acute (earliest) stage of HIV infection,^{162–164} but little follow-up work has been performed to address whether this mechanism is maintained for a considerable duration or whether T-cell output is quickly impaired following a short-lived initial burst. Other researchers have noted that the thymus, as it is infected and the cells therein depleted, eventually declines in function in HIV patients.^{165,166} Many HIV-infected individuals, compliance with HAART notwithstanding, do not experience ample reconstitution of the T-cell/TCR repertoire.¹⁶⁷ The function of S1P in the egress of mature thymocytes during HIV infection has not been examined. S1P and its receptors, if found to be present and functional during HIV infection, could represent a tantalizing new possibility for HIV therapy if the system were able to be modulated to promote enhanced thymic output and controlled so that only mature T cells would egress and repopulate the periphery.

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ABBREVIATIONS

BLT	Bone marrow/liver/thymus
HIV	human immunodeficiency virus
HSC	hematopoietic stem cell
KLF2	Kruppel-like factor 2
NK	natural killer
RTE	recent thymic emigrants
SCID	severe combined immunodeficiency
S1P-R1	sphingosine-1-phosphate receptor 1
TCR	T-cell receptor
thy/liv	thymus/liver

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FIG. 1.
Expression of cell-surface and intracellular markers on thymocytes across developmental stages. *Yellow*, Thymic cortex; *brown*, medulla.

S1P-R1 expression in the thymus

FIG. 2.

Expression of S1P-R1 across developmental stages in the human thymus.

FIG. 3.
Migration of mature naïve T cells toward S1P in the blood.

FIG. 4. Schematic diagram of the generation of humanized bone marrow/liver/thymus (BLT) mice used in HIV research.