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Role of secondary lymphoid tissues in primary and memory T-cell responses to a transplanted organ

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Abstract

Secondary lymphoid tissues are the hub of adaptive immune responses wherein rare cognate lymphocytes encounter dendritic cells bearing antigen from peripheral tissues and differentiate into effector and memory cells that eliminate antigen. It is accepted that immune responses against microbial and tumor antigens are initiated within secondary lymphoid tissues. There is less agreement on whether the same principle applies to immune responses to a transplanted organ because an allograft expresses foreign major histocompatibility complex and contains donor antigen presenting cells that could activate T cells directly in situ leading to rejection. Recent studies confirm that although naïve T cells can be primed within the allograft, their differentiation to effect rejection is dependent on secondary lymphoid tissues. Antigen-experienced memory T cells, unlike Naïve T cells, function largely independent of secondary lymphoid tissues to cause allograft rejection. In an alloimmune response, secondary lymphoid tissues support not only immune activation but also immune regulation essential for allograft survival. Here, we will review recent findings and discuss the role of secondary lymphoid tissues in primary and memory alloimmune responses.

1. Introduction

Naïve T cells exist in frequencies as few as 1 in 10^5 for a single antigen and are found predominantly in secondary lymphoid tissues (spleen, lymph nodes [LNs], and mucosal associated lymphoid tissues) [1,2]. The organized anatomical niches and chemokine milieu of secondary lymphoid tissues facilitate interactions between rare cognate lymphocytes and antigen-bearing dendritic cells (DCs) leading to productive adaptive immune responses [3–5]. Secondary lymphoid tissues support maturation and survival of naïve T cells, and their differentiation into effector and memory T cells that eliminate the inciting antigen in peripheral tissues leading to protective immunity [6–8]. In the absence of secondary lymphoid tissues, immune responses against microbial antigens and tumors are impaired resulting in host mortality [9,10]. Although the significance of secondary lymphoid tissues is well recognized in the initiation of antitumor and antimicrobial immune responses, it remains an area of contention in immune responses to transplanted organs [11]. Several features distinguish

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immune responses to a transplanted organ from that of immune responses to other foreign antigens: (*a*) recipient T cells recognize donor major histocompatibility complex (MHC) with or without antigenic peptide directly (direct allorecognition) in addition to classic self-MHC restricted recognition of alloantigenic epitopes (indirect allorecognition) [12–14]; (*b*) T cells that recognize foreign MHC antigens exist in extraordinarily high frequencies of 1 in 10 to 1 in 100 compared to relatively rare frequencies of T cells that recognize nominal antigens (1 in 10^5) [15]; (*c*) an allograft is subjected to ischemia-reperfusion injury during transplantation and depicts a complex inflamed tissue expressing foreign MHC and containing resident donor antigen presenting cells (APCs) that can migrate into recipient secondary lymphoid tissues; and (*d*) unlike inbred mice, humans harbor high frequencies of alloreactive memory T cells arising from either prior sensitization events or heterologous infections [16–18]. Thus, a transplanted organ represents a unique milieu of foreign antigen that underscores the need for understanding how alloimmune responses arise to effectively target allograft rejection. Here, we will review recent findings and discuss the role of spleen and LN secondary lymphoid tissues in primary and memory alloimmune responses leading to graft rejection.

2. Secondary lymphoid tissues

Spleen, LNs, and mucosal associated lymphoid tissues constitute secondary lymphoid tissues that are located strategically to efficiently trap foreign antigens entering via bloodstream, peripheral tissues, and mucosal sites, respectively. The microarchitecture of all secondary lymphoid tissues facilitates interactions between antigen-bearing DCs, B cells, and T cells to initiate adaptive immune responses. Despite these common features, differences in routes of antigen transport, lymphocyte trafficking, and unique cell populations determine the role of a specific secondary lymphoid tissue in immune responses to various foreign antigens including transplanted organs (Table 1). Mucosal-associated lymphoid tissues will not be discussed here.

2.1. Spleen

Spleen is the largest blood-filtering organ and plays an important role in immune responses to blood-borne antigens. Blood entering the spleen from afferent splenic artery percolates through branching arterioles into the marginal sinus and drains into venous sinuses of the red pulp [19]. Blood-borne antigens, DCs, and lymphocytes entering the spleen pass through the marginal zone containing marginal zone metallophilic macrophages, marginal zone macrophages, and marginal zone B cells before reaching the lymphoid white pulp containing T-cell rich periarteriolar lymphoid sheath (PALS) and B-cell follicles [20,21]. Fibroblastic reticular cell (FRC) networks provide the framework for migration of T and B cells into white pulp [22]. CCL19 and CCL21 on FRCs guides migration of CCR7 expressing T cells into PALS [22]. Similarly, CXCL13 on FRCs prompts migration of CXCR5 expressing B cells into B-cell follicles [22–25]. Upon encountering specific antigen, T and B cells undergo activation and down-regulate CCR7 and CXCR5, respectively, resulting in their exclusion from PALS and B-cell follicles in white pulp to reenter bloodstream [22–24]. Concomitant with down-regulation of CXCR5, developing plasmablasts up-regulate CXCR4 and localize to red pulp expressing CXCL12 where secreted antibodies can have immediate access to circulation [26].

Marginal zone metallophilic macrophages, marginal zone B cells, and B1 B cells are cell populations that are unique to spleen [21]. Marginal zone metallophilic macrophages express pattern recognition receptors SIGNR1, MARCO, and SIGLEC1 that are crucial for a rapid response to blood-borne pathogens such as *Mycobacterium tuberculosis*, *Escherichia coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Neisseria meningitides* [27–31]. Marginal zone metallophilic macrophages are the main producers of interferon α and interferon β essential for viral clearance and absence of this population after LCMV infection leads to disseminated disease [32–34]. Marginal zone B cells are rapid producers of immunoglobulin

M after exposure to blood-borne antigens, shuttle antigen to follicular DCs (FDCs) in follicles, and also function as APCs for T-cell activation [35–37]. B1 B cells recognize carbohydrate antigens and produce antibodies against capsular polysaccharides of pneumococcus and blood group antigens [38–40]. These unique cell populations confer protective immunity mediated by spleen and hence the life-long risk of fatal infections after splenectomy necessitating vaccinations and prophylactic antibiotics [41]. In transplantation, elimination of the spleen resident cell populations by splenectomy enables ABO-incompatible graft survival and management of antibody-mediated rejection in sensitized patients [42,43].

2.2. Lymph nodes

Peripheral tissues are drained by afferent lymphatics that converge in LNs [44]. Lymph-borne antigen and DCs from afferent lymphatics enter subcapsular sinus and pass through cortex (B-cell rich), paracortex (T-cell rich), and medullary cords (plasma cell-rich) via cortical and medullary sinuses before draining into efferent lymphatics [45,46]. Lymphocytes enter the LNs through specialized high endothelial venules (HEV) [47,48]. Adhesion molecules, peripheral node addressin (PNAd), and CCL21 expressed on HEV promote arrest and homing of T and B cells bearing CD62L (L-selectin) and CCR7, respectively [5,49,50]. CCR7 expressing T cells and DCs migrate via fibroblastic reticular cell networks to CCL19- and CCL21-rich paracoritcal areas [51,52]. CXCR5-expressing B cells migrate via follicular DC networks to CXCL13-rich cortical areas [52]. Interactions between antigen-bearing DCs and cognate B and T cells lead to extensive proliferation, differentiation, and formation of germinal centers [53–55]. Lymphocytes down-regulate sphingosine-1-phosphate receptor 1 and exit LNs through efferent lymphatic vessels and enter the bloodstream via thoracic duct [56,57].

Subcapsular sinus macrophages are unique to LNs and are crucial in trapping antigens entering via lymphatics and limiting their systemic spread [58]. These macrophages present antigen to B cells and drive B-cell activation [58,59]. Tissue-derived DCs such as Langerhans cells from skin and lung DCs have access to LNs via afferent lymphatics but not to the spleen. Lymph node stromal cells have immunoregulatory properties and present endogenously expressed antigens leading to deletional tolerance of self-reactive CD8 T cells [60]. In the absence of draining LNs, immune responses to tissue invading pathogens are disrupted leading to dissemination or breakdown of tolerance to intestinal antigens [58,61,62].

2.3. Allografts and secondary lymphoid tissues

The location of antigen and route of transport to secondary lymphoid tissues determine the importance of spleen and LNs in the resulting adaptive immune response and protective immunity [58,63]. Lymph nodes are essential for control of subcutaneous tumors, whereas spleen is essential to mount an immune response after intravenous immunization for control of lung metastases [63]. Antigen from the transplanted organ arrives in secondary lymphoid tissues in the form of donor DCs migrating from the graft, recipient DCs carrying donor antigens, and as exosomes and soluble antigens derived from the graft [64]. Antigen-bearing DCs enter spleen exclusively via the bloodstream, whereas LNs are entered via both the bloodstream and lymphatics [65–68]. Tissue-derived DCs such as epidermal Langerhans cells, dermal DCs, and lung DCs carry antigen via draining lymphatics into LNs and do not have access to spleen unless migrating DCs are not retained in the draining LN, exit via efferent lymphatics into circulation, and subsequently enter the spleen [63,67,69–72]. The role of LN vs spleen in an alloimmune response is primarily dependent on how antigen is carried to secondary lymphoid tissues, which is in turn determined by whether the transplanted organ has lymphatic drainage or not. Skin allografts are transplanted onto recipient dermal beds that drain by afferent lymphatics into LNs and undergo de novo neovascularization [73]. Interruption of lymphatic drainage from skin allografts either significantly delayed or prevented graft rejection, suggesting that LNs are the main site of the alloimmune response to skin allografts

[74,75]. In the absence of draining lymphatics, skin allografts are eventually rejected [76] suggesting that antigen from neovascularized skin allografts is possibly carried by blood-derived DC or in soluble form or as exosomes eliciting an immune response that led to rejection because skin-derived DCs migrate via lymphatics and do not have direct access to spleen [69,70]. In the case of primarily revascularized transplanted organs such as heart and kidney, draining lymphatics from the graft are disrupted during transplantation and the route of antigen transport to secondary lymphoid organs is mainly via bloodstream and, hence, spleen is the main site of alloimmune response [77,78]. Alloimmune response to pancreatic islet allografts transplanted under the kidney capsule that are drained by lymphatics and neovascularized is elicited in LNs [79]. When pancreatic islet allografts are transplanted intraportally in the liver with access to circulation, the ensuing alloimmune response occurs in the spleen [79]. Thus, draining LNs serve as the primary site of immune response for allografts with lymphatic drainage and spleen is the site of immune responses to primarily revascularized allografts or allografts that have access to circulation.

3. Secondary lymphoid tissues and primary alloimmune responses

Naïve T cells responding to a foreign antigen circulate preferentially through secondary lymphoid tissues and gain access to nonlymphoid tissues such as allografts only after differentiation into effector or memory T cells [80–82]. Activation and differentiation of naïve T cells into effectors requires encountering specific antigen (signal 1) in the context of appropriate co-stimulatory signals (signal 2) on professional antigen-presenting cells such as DCs [83–85]. Such cognate interactions between antigen-bearing DCs and naïve T cells when occurring within organized secondary lymphoid tissues will lead to T-cell activation and protective immunity against tumor or microbial antigens [86–88]. Antigen encountered by naïve T cells in peripheral tissues without access to secondary lymphoid tissues fails to induce a productive immune response and leads to ignorance [10,86].

3.1. Primary alloimmune responses in the absence of secondary lymphoid tissues

The fundamental question of whether an alloimmune response originates in secondary lymphoid tissues or occurs within the graft itself has troubled transplant biologists for decades [11,89]. Allograft rejection is recognized as a T cell–dependent process [90]. Disrupting lymphatic drainage from the transplanted graft to LNs prevented rejection of skin allografts despite preserved circulation via an intact vascular pedicle [74]. This suggested that T-cell activation in response to a skin allograft occurred primarily within LNs. However, when rejection of vascularized kidney allografts that lacked lymphatic drainage was observed, it led to the notion that T cells were activated within the graft itself (peripheral sensitization) in organs undergoing primary revascularization during transplantation [78,91,92]. Since then, multiple investigators have tackled the question of whether secondary lymphoid organs are required for alloimmune responses using mice with disrupted lymphoid organogenesis [93–95]. Lymphoid organ development is initiated when lymphotoxin (LT) $\alpha 1\beta 2$ on circulating cells trigger LTβR in stromal cells activating NF-κB–inducing kinase (NIK) and NF-κB, leading to production of chemokines CCL19, CCL21, CXCL12, and CXCL13 and recruitment of lymphoid cells [96]. Lymphoid organogenesis is disrupted in LTα-deficient, LTβR-deficient and alymphoplastic (*aly/aly*) mice that have a mutation in the gene encoding NIK [97–100]. These mice lack peripheral LN, mucosal-associated lymphoid tissues, and upon splenectomy, lack all secondary lymphoid organs and, hence, are extensively used as tools to study the role of secondary lymphoid organs in immune responses including allograft rejection.

Lakkis et al [93] examined skin and heart allograft ($H\text{-}2^k$) rejection in *aly/aly* ($H\text{-}2^b$), asplenic homeobox 11 (*Hox11*−/−) deficient, and splenectomized *aly/aly* mice. Skin allograft acceptance of more than 100 days in *aly/aly* mice lacking peripheral LNs confirmed prior findings that skin allograft rejection was dependent on lymphatics and draining LNs [93].

Vascularized heart allografts were rejected in *Hox11*−/− and *aly/aly* mice but not in splenectomized *aly/aly* mice even after 160 days after transplantation [93]. Transfer of lymph node cells from allosensitized but not naïve *wt* mice precipitated heart allograft rejection in splenectomized *aly/aly* mice [93]. These results showed that initiation of alloimmune response requires secondary lymphoid tissues and that either LNs or spleen were sufficient to cause vascularized allograft rejection. T cells from splenectomized *aly/aly* recipients of heart transplants precipitated allograft rejection in T cell–deficient TCRβδ−/− hosts, suggesting that tolerance had not developed and T-cell function was intact [93]. Thus, alloantigens were ignored by naïve T cells unless they were encountered within secondary lymphoid tissues to undergo activation and cause allograft rejection. Zhou et al showed that heart allografts (H-2^d) in splenectomized LTα$^{-/-}$ (MST 50 days) and LTβR$^{-/-}$ mice (5/6 mice rejected between 60 and 87 days) were eventually rejected despite the absence of secondary lymphoid tissues unlike the indefinite allograft survival observed in splenectomized *aly/aly* mice [95]. Also, Wang et al [94] reported that heart allograft (H-2^k) survival in splenectomized LTα$^{-/-}$ mice (MST > 99 days) was prolonged. T-cell activation after intestinal allograft transplantation occurred mainly in donor mesenteric lymph nodes and either recipient or donor lymphoid organs were sufficient to cause rejection [94]. It is possible that differences in allograft donor strains and intrinsic abnormalities in addition to defects in lymphoid organogenesis in LTα$^{-/-}$ LTβR$^{-/-}$ and *aly/aly* mice contributed to the disparities in allograft survival observed in these studies. LTα$^{-/-}$ and LTβR$^{-/-}$ mice are characterized by perivascular CD4 T- and B-cell infiltrates in nonlymphoid tissues, and it is unclear whether such infiltrates represent naïve or activated cells [99,100]. Heart allograft rejection in splenectomized LTα$^{-/-}$ and LTβR$^{-/-}$ mice is characterized by dominant CD4 T-cell infiltrates [95], raising the possibility that abnormal T-cell infiltration into nonlymphoid tissues could have contributed to eventual allograft rejection in these hosts. Because NIK is also a mediator of NF-κB activation, impaired B-cell or macrophage function, in addition to lack of secondary lymphoid organs, could have contributed to allograft survival in splenectomized *aly/aly* mice [101]. Despite these intrinsic differences in LTα$^{-/-}$, LTβR$^{-/-}$ and *aly/aly* mice, it is clear that primary alloimmune responses to skin, heart, and intestinal allografts are significantly impaired in the absence of all secondary lymphoid tissues. Similarly, initiation of GVHD after bone marrow transplantation is also dependent on secondary lymphoid tissues [102].

Alloimmune responses to lung allografts, like immune responses against respiratory viruses, defy the requirement for secondary lymphoid tissues [103,104]. Lung tissue microenvironment is rich in DCs and expression of PNAd, CCL21, and CXCL13 is induced under inflammatory conditions resulting in the recruitment of T and B cells and formation of inducible bronchial associated lymphoid tissue that facilitate immune responses in situ [105]. Gelman et al show that lung allografts from *aly/aly* donors (H-$2^{k/b}$) transplanted to splenectomized *aly/aly* recipients activate T-cells within the allograft leading to rejection independent of all secondary lymphoid tissues. PNAd expression and FDCs were not found within the allografts to support fully formed inducible bronchial associated lymphoid tissue [103]. However, it is not clear if CCL21 (constitutive and inducible) and CXCL13 (inducible) were expressed in the transplanted lung allografts because these chemokines are induced independent of LTα that could lead to recruitment of lymphocytes into the lung, their organization into lymphoid-like structures and subsequent activation by resident DCs [105].

3.2. Allorecognition and primary alloimmune responses outside secondary lymphoid tissues

Impaired heart allograft rejection in the absence of secondary lymphoid tissues implied that naïve T cells recognize alloantigens presented on either recipient or donor APCs within these tissues to differentiate into effectors that subsequently migrate into the allograft causing rejection. However, peripheral sensitization proposed 40 years ago suggests that recipient lymphocytes recognize alloantigens within the graft itself, possibly on the vascular

endothelium and home to lymphoid tissues to cause rejection [89,91,92]. Graft endothelial cells have been recognized to recruit leukocytes, promote DC differentiation, and support activation and differentiation of T cells [106,107]. Endothelial cells also express MHC I and II, co-stimulatory molecules (eg, CD80), and can function as APCs to directly activate T cells [108]. Kreisel et al provided in vivo evidence of allorecognition by CD8 T cells within the allograft via the direct pathway of antigen presentation [106]. Using bone marrow transplantation, chimeric allografts were created that expressed donor-type MHC on parenchymal cells including endothelial cells, and expression of recipient type MHC was limited to hematopoietic APCs. Such chimeric allografts were transplanted into CD4-depleted recipients containing CD8 TCR-tg T cells that were capable of recognizing only donor MHC I and underwent acute rejection [106]. Thus, direct allorecognition of donor MHC expressing nonhematopoietic cells such as endothelial cells can occur within the allograft, whereas indirect allorecognition might be restricted to secondary lymphoid organs [106]. However, it was not tested whether T cells that recognized alloantigens directly within the allograft differentiated into effectors in situ or in secondary lymphoid organs. Impaired alloimmune responses to heart allografts in hosts lacking secondary lymphoid tissues suggest that despite allorecognition within the graft, differentiation into effectors that cause allograft rejection is still dependent on secondary lymphoid tissues. These studies together support peripheral sensitization, suggesting that T cells can recognize alloantigen within the graft itself and differentiate in lymphoid tissues to mount a rejection response.

Bone marrow has been reported to serve as the priming site outside secondary lymphoid tissues for T and B cells responding to blood borne antigens [109]. Transgenic T cells respond to intravenous ovalbumin in the bone marrow of splenectomized *aly/aly* mice and generate immunologic memory [109]. However, in primary alloimmune responses, bone marrow is unlikely to be the site of activation of T cells responding to either organ allografts or bone marrow grafts because allograft rejection was impaired in the absence of secondary lymphoid tissues despite an intact bone marrow compartment [93–95,102]. Other sites of naïve T-cell activation outside secondary lymphoid tissues include tertiary lymphoid organs (TLOs) present in many chronic inflammatory states such as autoimmunity (Hashimoto thyroiditis, rheumatoid arthritis, multiple sclerosis, etc), infectious diseases (chronic hepatitis C, chronic lyme disease, etc), and allograft rejection [110–113]. Tertiary lymphoid organs are ectopic lymphoid cell accumulations in nonlymphoid tissues that resemble peripheral lymph nodes. TLOs contain discrete T- and B-cell populations, FDC networks, and HEV that express adhesion molecules PNAd and MAdCAM-1 to recruit CD62L and $\alpha_4\beta_7$-bearing naïve lymphocytes [110]. Nasr et al have shown that TLOs form in donor grafts transplanted into splenectomized *aly/aly* mice and support differentiation of naïve T cells to effector and memory T cells that cause allograft rejection [111]. Thus, in addition to secondary lymphoid tissues, TLOs in allograft tissues, can serve as sites of primary alloimmune responses that lead to allograft rejection.

4. Secondary lymphoid tissues and immune regulation of alloimmune responses

In an alloimmune response, secondary lymphoid organs are the site of not only immune activation but also immune regulation that leads to allograft tolerance. Location, dose and duration of antigen exposure, and state of maturation of APC determine whether T-cell activation or tolerance will emerge [114]. Antigen that is sequestered within peripheral tissues and presented to naïve T cells outside the context of secondary lymphoid tissues is ignored and will lead to neither activation nor tolerance of T cells [9,10,93,115,116]. Although spleen or LNs are sufficient to mediate allograft rejection [93,95], the primary site of immune regulation leading to allograft tolerance varies between spleen and LNs depending on the organ transplanted. Also, based on the primary route of antigen transport, antigen load in spleen vs LNs could vary between different transplanted organs, that is, draining LNs possibly have

higher antigen load than spleen after skin transplantation, whereas antigen load in spleen could be higher than in LNs after heart transplantation. The antigen load in specific secondary lymphoid organs could determine whether a productive or abortive immune response develops resulting in T-cell activation or T-cell regulation within the specific secondary lymphoid tissue [117]. Thus, within the same immune response, T-cell activation and regulation could be seen in secondary lymphoid tissues.

In the case of skin allografts that are drained by lymphatics into LNs, the primary site of the alloimmune response is the draining LNs [74]. Spleen does compensate for the absence of LN in $LT\alpha^{-/-}$ and $LT\beta R^{-/-}$ mice, and an immune response to skin allografts in the spleen leads to rejection in the absence of LNs. However, in wild-type recipients that are splenectomized, accelerated skin allograft rejection is observed in the absence of a spleen [118]. Similarly, immune response to corneal allografts occurs primarily in LNs; and rejection is delayed in the absence of LNs, whereas it is accelerated in the absence of spleen [119]. Spleen is essential for anterior chamber–associated immune deviation promoting survival of corneal allografts [120]. Development of regulatory T cells contributing to anterior chamber–associated immune deviation is dependent on splenic B cells, MZ B cells, γδT cells, and NK T cells [121,122]. These findings suggest that in allografts that are drained by lymphatics and where LNs are the primary site of immune activation leading to rejection, spleen seems to be the site of immune regulation. It remains to be determined whether it is soluble antigen or the maturation state of tissue-derived antigen-bearing APCs reaching the spleen or unique spleen resident cell populations such as B1 B cells or marginal zone B cells that determine the emergence of immune regulation at this site.

Tolerance to vascularized heart allografts has been reported to occur in LN via the indirect pathway of alloantigen presentation to T cells under tolerizing conditions [123,124]. Donor APCs from vascularized heart allografts enter via bloodstream into the spleen, the site of alloimmune activation causing rejection of heart allografts [77]. Antigen-bearing APCs from vascularized heart allografts can access LNs only via HEV because lymphatic drainage is disrupted during transplantation. APC–T-cell interactions in the LN are essential for immune regulation and tolerance to heart allografts [125]. Blocking LN homing led to abrogation of tolerance resulting in heart allograft rejection [125]. Antigen presentation by recipient plasmacytoid DCs (pDCs) in the LN and not the spleen gave rise to regulatory T cells after allogeneic heart transplantation [124]. Although pDCs were found in the spleen as well in alloimmue responses leading to either rejection or tolerance, pDC and T-cell interactions specifically in the LN and not the spleen led to development of regulatory T cells [14,124]. It is possible that optimal amounts of antigen entering mainly the spleen after heart transplantation lead to immune activation, whereas limited amounts reaching the LN resulted in formation of regulatory T cells. Other possibilities that remain to be examined are whether tissue-derived DC populations that can access LNs via lymphatics or LN stromal cells contribute to the emergence of immune regulation at this site.

5. Secondary lymphoid tissues and memory alloimmune responses

Naïve T-cell circulation is restricted to secondary lymphoid tissues, whereas memory T cells migrate broadly through lymphoid and nonlymphoid tissues [81,82,126]. Unlike naïve T cells, memory T cells have less stringent activation requirements and can thus interact with foreign antigen at the site of its location in peripheral tissues without requiring co-stimulatory help from licensed APCs in secondary lymphoid tissues [127,128]. Memory T cells underwent activation and effectively cleared influenza virus in the absence of secondary lymphoid tissues [104]. Similarly, memory B cells produced neutralizing antibodies after rechallenge with influenza virus and LCMV in hosts lacking secondary lymphoid tissues [104,129]. Unlike inbred mice, patients who are recipients of organ allografts often harbor memory T cells

resulting from either previous allogeneic sensitization events (blood transfusions, prior organ transplants, etc) or heterologous immunity [18,130,131]. Because immunosuppressive drugs in clinical use such as FTY720 modulate lymphocyte trafficking from secondary lymphoid tissues, it is vital to understand how these tissues influence development and activation of memory T cells responding to transplanted organs.

5.1. Secondary lymphoid tissues and development of memory T cells

Induction of a primary alloimmune response and activation of naïve T cells to generate effectors that cause allograft rejection are largely dependent on secondary lymphoid tissues [93–95]. After activation, naïve T cells give rise to effector T cells that can migrate into peripheral tissues and mediate allograft rejection in hosts lacking secondary lymphoid tissues [93]. Whether effector T cells that egress into peripheral tissues can differentiate to memory T cells within these tissues or require secondary lymphoid tissues to form memory T cells was addressed by Obhrai et al [132]. Sorted effector CD4 and CD8 T cells were obtained from allosensitized mice, transferred into naïve congenic splenectomized *aly/aly* recipients, and their differentiation to memory T cells was tested in comparison to wild-type adoptive hosts [132]. Effector CD8 but not CD4 T cells proliferated and differentiated into functional memory T cells that rejected skin allografts in splenectomized *aly/aly* recipients [132]. In the absence of secondary lymphoid tissues, effector CD4 T cells proliferated poorly and did not survive to generate memory T cells [132]. Providing extended access to secondary lymphoid tissues rescued proliferation and differentiation of effector CD4 T cells to memory T cells in hosts lacking all secondary lymphoid tissues [132]. Thus, CD4 but not CD8 T cells depend on secondary lymphoid tissues to develop into memory T cells [8,132]. It remains to be elucidated which key cell populations and/or cytokines within the microenvironment of secondary lymphoid tissues support CD4 T cells differentiate into memory T cells.

5.2. Memory alloimmune responses in the absence of secondary lymphoid tissues

Memory T cells circulate broadly through both lymphoid and nonlymphoid peripheral tissues. We tested whether memory T cells can function to reject an allograft, a peripheral tissue, in the absence of secondary lymphoid tissues. Memory T cells from allosensitized wild-type mice were enriched for alloreactive CD8 T cells and transferred into splenectomized *aly/aly* mice that underwent allogeneic heart transplants [133]. Unlike naïve T cells, memory T cells were able to cause acute rejection of cardiac allografts in hosts lacking all secondary lymphoid tissues [133]. Similarly, either CD4 or CD8 memory T cells sorted from allosensitized mice were able to mediate rejection of skin allografts in splenectomized *aly/aly* mice [132]. In addition, CD4 and CD8 memory T cells proliferated and were maintained for 12 weeks in splenectomized *aly/aly* adoptive hosts comparable to wild-type mice [132]. Thus, once generated, memory T cells can function and persist long term independent of secondary lymphoid tissues, an important advantage over naïve T cells that contributes to the rapidity of memory responses.

Memory T cells are heterogeneous and consist of 2 populations of cells with distinct homing patterns and effector functions: (*a*) central memory T cells (T_{CM}) express lymphoid homing receptors CD62L and CCR7, home preferentially to secondary lymphoid tissues and have delayed effector function; and (*b*) effector memory T cells (T_{EM}) lack expression of lymphoid homing receptors, CCR7 and CD62L, home predominantly to peripheral tissues and have immediate effector function [134,135]. Because a transplanted organ is a peripheral tissue, we tested whether CD8 T_{EM} have an advantage over CD8 T_{CM} in allograft rejection in splenectomized *aly/aly* adoptive hosts that lack all secondary lymphoid tissues. In the absence of secondary lymphoid tissues, skin allograft rejection in adoptive hosts of CD8 T_{CM} was significantly delayed compared to that in recipients of T_{EM} [136]. CD8 T_{CM} required secondary lymphoid tissues for their activation and differentiation into effectors to migrate into skin allografts [136]. Albeit delayed, skin allografts were eventually rejected in splenectomized

aly/aly recipients of CD8 T_{CM}, suggesting that both CD8 T_{CM} and T_{EM} can function outside secondary lymphoid tissues [136]. It remains to be tested whether central and effector memory T cells can function similarly in responses to vascularized transplants such as heart or kidney allografts. FTY720 (sphingosine-1-phosphate receptor agonist) is in clinical use as an immunosuppressive agent and prevents egress of lymphocytes from secondary lymphoid tissues [137]. FTY720 preferentially affects naïve T cells and T_{CM} that predominate secondary lymphoid tissues but not T_{EM} [138]. FTY720 treatment of cardiac allograft recipient mice harboring CD4 memory T cells led to delayed allograft rejection [138]. With FTY720 treatment, although CD4 memory T cells accumulated in secondary lymphoid tissues, their numbers persisted in peripheral tissues comparable to untreated mice, suggesting that these unaffected CD4 T_{EM} possibly contributed to eventual rejection of the allograft [138]. Despite sequestration within secondary lymphoid tissues, alloantibody production and CD8 T-cell responses were intact, suggesting that function of CD4 memory T cells was not inhibited [138]. These studies illustrate the complexity and resilience of memory T cells that should be considered carefully when designing therapeutic strategies targeting memory T cells to inhibit allograft rejection.

6. Summary

A fundamental question, whether an alloimmune response is initiated within the allograft or within secondary lymphoid tissues, has yielded complex answers that underscore the importance of context and diversity in adaptive immune responses. It is now clear that T cells can recognize alloantigens via the direct pathway of antigen presentation within the allograft. Antigen recognition by naïve T cells outside secondary lymphoid tissues is possibly restricted to alloimmune responses because migration of naïve T cells into tissues is limited and alloreactive T cells exist in high frequencies. However, subsequent differentiation of naïve T cells into effectors that cause rejection of skin, heart, and intestinal allografts depends on secondary lymphoid tissues and is similar to other adaptive immune responses. These findings taken together support Medawar and Gowans' "peripheral sensitization" process where circulating lymphocytes recognize alloantigens within the allograft and initiate a reaction against the graft after they have homed to spleen and LNs. Lung is a unique peripheral tissue wherein an alloimmune response is initiated in situ, analogous to immune responses against respiratory infections, and culminates in graft rejection independent of secondary lymphoid tissues. In sensitized recipients harboring memory T cells, allograft rejection can occur independent of secondary lymphoid tissues. Therapeutic strategies aimed to target lymphocyte trafficking should inhibit both lymphoid tissue resident T_{CM} and peripheral tissue resident T_{EM} to be effective in preventing graft rejection. Because secondary lymphoid tissues also support development of regulatory T cells, better understanding of the role of specific secondary lymphoid tissues in immune regulation of responses to different transplant organs could provide novel strategies for targeting allograft rejection.

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Table 1

Distinguishing features of secondary lymphoid tissues

	Spleen	Lymph node
Antigen entry	Via bloodstream	Via lymphatics draining interstitial spaces of peripheral tissues
Site of antigen entrapment	Marginal zone	Subcapsular sinus
Antigen passage route	Marginal sinus–FRC network–PALS/B-cell follicles	Subcapsular sinus–FRC network–paracortex/cortex
T-cell area	PALS	Paracortex
B-cell area	B-cell follicles	Cortex
Lymphocyte traffic	Marginal zone–FRC network–PALS/B-cell follicles–venous sinuses	HEV–FRC/FDC nerwork–paracortex/cortex–efferent lymphatics
Unique cell populations	Marginal zone metallophilic macrophages, marginal zone B cells, B1 B cells	Subcapsular sinus macrophages, LN stromal cells