ELSEVIER

Seminars in Immunology 20 (2008) 137–146

seminars in
IMMUNOLOGY

www.elsevier.com/locate/ysmim

Review

Lymphoid tissue engineering: Invoking lymphoid tissue neogenesis in immunotherapy and models of immunity

Darrell J. Irvine [a,b,*], Agnieszka N. Stachowiak [a,b], Yuki Hori [a]

[a] *Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*
[b] *Department of Materials Science & Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

Abstract

The plasticity of the immune system is evident in the reorganization of secondary lymphoid organs during immune responses, lymphoid tissue neogenesis occurring during chronic inflammation or graft rejection, and the engineered lymphoid tissue formation induced by ectopic expression of single lymphoid tissue-associated genes. Approaches seeking to harness this plasticity for immunotherapy are under investigation, particularly by controlling immune cell recruitment and lymphoid tissue formation at tumor sites. By combining strategies from ectopic tissue induction models with methods from tissue engineering, new approaches for studying lymphoid tissue development and immunotherapy may be possible.

Keywords: Tissue engineering; Lymphoid tissue neogenesis; Tertiary lymphoid tissues; Homeostatic chemokines; Lymphotoxin

1. Introduction

The field of tissue engineering (or regenerative medicine) is broadly concerned with the regeneration of tissue damaged by disease or trauma, and the development of *in vitro* tissue models that enable mechanistic studies of tissue function [1–3]. Tissue engineering strategies typically employ an artificial or natural extracellular matrix as a scaffold for tissue formation, which may be preloaded with cells or factors prior to injection/implantation at a target site. This matrix serves to replace damaged/lost ECM, provide a defined space and mechanical support for tissue formation, and often contains cues (soluble, matrix-bound, or released over time) that guide tissue differentiation and/or support vascularization and recruitment of host cells to populate the neotissue [4–12]. These approaches have been applied to construct a wide variety of tissues including bone [6,13], blood vessels [2,14], skin [15,16], liver [17], cartilage and muscle [18–22], and tissues of the peripheral and central nervous systems [23–26].

One key challenge faced in many of these applications is that the tissues of interest often have limited plasticity or potential to regenerate/self-organize in the adult (in some cases, perhaps none at all). This situation stands in striking contrast to the plasticity of the immune system, where secondary lymphoid organs (SLO, such as the lymph nodes, spleen, and Peyer's patches in the gut) undergo major reorganization in the course of 'routine' immune responses, and where *de novo* ectopic lymphoid tissue formation ('tertiary' lymphoid tissue) is known to occur in certain disease and inflammatory settings [27,28]. Further, studies with transgenic mouse models have demonstrated that 'lymphoid tissue engineering' can be achieved via the constitutive expression of single factors at an ectopic site. This striking malleability of the adult immune system, if combined with engineering approaches developed in the field of tissue engineering, might provide important new avenues for the controlled formation of tertiary lymphoid tissue. Such an approach at the interface of immunology and tissue engineering could provide a new paradigm for controllably inducing lymphoid tissue *in vivo*, both for understanding lymphoid organ development and neogenesis in the adult, and for site-directed lymphoid tissue neogenesis for immunotherapy, particularly in the treatment of cancer.

Here we will describe the organogenesis and plasticity of secondary lymphoid organs (SLO) and review current data that explores the concept of lymphoid tissue engineering in model systems and immunotherapy. We will first summarize the current understanding of critical cues in lymphoid organ development and tertiary lymphoid tissue formation induced by ectopic cytokine/chemokine expression or during chronic inflammation and graft rejection. The application of key sig-

* Corresponding author at: MIT Room 8-425, 77 Massachusetts Avenue, Cambridge, MA 02139, USA. Tel.: +1 617 452 4174; fax: +1 617 452 3293.
E-mail address: djirvine@mit.edu (D.J. Irvine).

1044-5323/$ – see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.smim.2007.10.010

nals from development to drive lymphocyte accumulation and in some cases overt lymphoid tissue neogenesis at tumor sites to combat cancer will then be discussed. Finally, recent studies that bring together concepts from tissue engineering and studies of lymphoid tissue neogenesis will be highlighted, and prospects for such approaches in the future will be considered.

2. Lymphoid tissue organogenesis: cues from development for neogenesis in the adult

Many of the cytokine and chemokine signals involved in lymphoid organogenesis during development have been found to also play important roles in tertiary lymphoid tissue neogenesis during immune responses/diseases and during experimental/therapeutic lymphoid tissue induction in the adult [29]. Secondary lymphoid organ development has been the subject of excellent recent reviews [27,28,30–32]; thus we will only summarize key facets germane to a discussion of therapeutic lymphoid tissue induction models.

Organogenesis of lymph nodes is believed to initiate with the development of lymphatics sprouting from blood endothelium, followed by colonization of lymph node sites by lymphoid tissue-inducer cells (LTICs), hematopoietic progenitors characteristically expressing CD45, CD4, interleukin-7 receptor-α (IL-7Rα), and lacking CD3 [33,34]. These cells interact with local VCAM-1$^+$ stromal cells, initiating a series of signaling cascades in the local environment [28,35]. Key among the early triggers driving lymphoid organ development are signals derived from two members of the tumor necrosis factor (TNF) superfamily, lymphotoxin-α and -β (LT-α and LT-β). Lymphotoxin-α (LT-α) functions either as a soluble homotrimer (LT-α_3) or as a heterotrimer (LT-$\alpha_1\beta_2$) formed by complexing with the cell surface membrane protein LT-β; these two forms of LT signal through distinct receptors. Signaling through IL-7Rα in LTICs triggers expression of LT$\alpha_1\beta_2$, which in turn engages lymphotoxin-β receptor (LT-βR) expressed on stromal cells [28,36]. LT-βR signaling is critical as it induces the expression of lymphoid organ-specific homeostatic chemokines (CCL19, CCL21, CXCL12 and CXCL13) [37,38] and leads to the induction of high endothelial venules (HEVs), the specialized blood vessels of secondary lymphoid organs [27]. HEVs induced by LT signaling express the adhesion molecules peripheral node addressin (PNAd) and/or mucosal addressin cell adhesion moelcule-1 (MAdCAM-1) that allow naïve lymphocytes to home to lymphoid tissues [39,40]. HEV induction supports the entry of naïve T and B cells into the lymphoid tissue, while expression of the B zone (CXCL13) and T zone (CCL19, CCL21, CXCL12) chemokines organizes the infiltrating cells into their respective areas of the organ [40–49].

The key role of LT signaling and homeostatic chemokines in lymph node development are revealed by studies in mice lacking one or more of these factors and mice where these genes have been transgenically expressed at ectopic tissue sites. Mice lacking LT-α, LT-β, or LT-βR lack lymph nodes [50–55]. Loss of CXCL13, a key chemoattractant for naïve B cells, or its receptor CXCR5, causes failure of most lymph nodes to form [48,49]. Using the rat insulin promoter (RIP) to create transgenic mice that specifically express cytokines in the pancreas and kidneys at time-points after development is normally complete [56–58], it was shown that local ectopic expression of LT-α or TNF-α resulted in a sustained inflammatory infiltrate of T cells, B cells, macrophages, and dendritic cells localized to the sites of transgene expression. Strikingly, in lymphotoxin-transgenic mice, these infiltrates were organized into distinct, segregated T cell- and B cell-rich clusters reminiscent of the segregated organization of T- and B-lymphocytes in SLO [58,59]. These ectopic lymphoid tissues were functional for generation of humoral immune responses and share many other features with ‘normal’ SLO: the same frequencies of B cells and ratio of CD4:CD8 T cells as normal lymph nodes, the presence of follicular dendritic cells, expression of homeostatic chemokines [60], and the presence of blood vessels with the unique morphology of high endothelial venules (HEVs) expressing the HEV markers PNAd and MAdCAM [56]. Organized infiltrates with characteristics of SLO are also induced in mice transgenically expressing CXCL13 or CCL21 under the RIP promoter (and to a lesser degree by CCL19 and CXCL12), in part due to LT expression induced by these chemokines as part of a positive feedback loop between chemokines and LT signaling [48,61–63]. Notably, the ability of constitutively expressed chemokines to induce lymphoid tissue formation depends on the tissue site, as expression of CCL21 induces organized lymphoid tissue when expressed in the pancreas or thyroid but not when expressed in skin [63–65]. From these data using transgenes to express cytokines and chemokines at ectopic tissue sites, it is clear that providing even a single cytokine constitutively can induce lymphoid tissue neogenesis, which provides a likely explanation for the lymphoid tissue-like structures observed in many situations of chronic inflammation/autoimmunity.

In addition to the critical role played by lymphotoxin and chemokines in organizing lymph nodes, other cytokines also play accessory roles of varying importance depending on the tissue site: For example, LIGHT (acronym for: ‘homologous to Lymphotoxins, shows Inducible expression, and competes with herpes simplex virus Glycoprotein D for HVEM, a receptor expressed by T lymphocytes’) contributes to the formation of mesenteric lymph nodes [66,67]. Mice lacking TNF-related activation-induced cytokine (TRANCE) or its receptor (TRANCER) lack lymph nodes, but Peyer’s patch and nasal-associated lymphoid tissue formation is intact [68–71]. These cytokines thus have unique, non-overlapping roles with the LT/TNF-α signals described above.

3. Lymphoid tissue neogenesis in disease settings

The native secondary lymphoid organs (lymph nodes, spleen, Peyer’s patches) exhibit substantial plasticity, undergoing major changes in their size, structure, cellular residents, and physical organization as part of normal immune responses [27]. Strikingly, the plasticity of the immune system appears to extend beyond the ‘dedicated’ SLO in adults. Though the normal process of lymphoid tissue organogenesis is stopped in the adult, several instances of tertiary lymphoid tissue formation during inflammation and graft rejection are known to occur. First,