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Review

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Review

Fulfilling Koch's postulates in glycoscience: HCELL, GPS and translational glycobiology

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Abstract

Glycoscience-based research that is performed expressly to address medical necessity and improve patient outcomes is called "translational glycobiology". In the 19th century, Robert Koch proposed a set of postulates to rigorously establish causality in microbial pathogenesis, and these postulates can be reshaped to guide knowledge into how naturally-expressed glycoconjugates direct molecular processes critical to human well-being. Studies in the 1990s indicated that E-selectin, an endothelial lectin that binds sialofucosylated carbohydrate determinants, is constitutively expressed on marrow microvessels, and investigations in my laboratory indicated that human hematopoietic stem cells (HSCs) uniquely express high levels of a specialized glycoform of CD44 called "hematopoietic cell E-/L-selectin ligand" (HCELL) that functions as a highly potent E-selectin ligand. To assess the role of HCELL in directing HSC migration to marrow, a method called "glycosyltransferase-programmed stereosubstitution" (GPS) was developed to custom-modify CD44 glycans to enforce HCELL expression on viable cell surfaces. Human mesenchymal stem cells (MSCs) are devoid of E-selectin ligands, but GPS-based glycoengineering of CD44 on MSCs licenses homing of these cells to marrow *in vivo*, providing direct evidence that HCELL serves as a "bone marrow homing receptor". This review will discuss the molecular basis of cell migration in historical context, will describe the discovery of HCELL and its function as the bone marrow homing receptor, and will inform on how glycoengineering of CD44 serves as a model for adapting Koch's postulates to elucidate the key roles that glycoconjugates play in human biology and for realizing the immense impact of translational glycobiology in clinical medicine.

Key words: exofucosylation, fucosyltransferase, mesenchymal stem cell, hematopoietic stem cell, cell migration

Introduction

Glycoscience is a field that broadly spans both chemistry and biology, distinguished by two principal types of investigators: glycochemists and glycobiologists. Within both groups, there exists a subset of investigators whose research is inspired by medical necessity and who practice glycoscience-based inquiry to address such need(s): I refer to these investigators as the "translational glycobiologists". I consider myself to be a translational glycobiologist. However, unlike most of my colleagues that have established their careers in glycoscience research, I was not mentored by any glycoscientist, nor was I formally trained in chemistry, enzymology, or structural biology. It was my deep interest in the problems faced by patients undergoing hematopoietic stem cell (HSC) transplantation that led me to appreciate the immense impact and potential of glycoscience within the practice of medicine.

As a second-year medical student (spring term, 1979), I had the opportunity to observe a bone marrow transplant (more accurately,

a "hematopoietic stem cell transplant") in a young male who was suffering from primary marrow failure (i.e., aplastic anemia). I was at the bedside when the filtered marrow cells were infused into the bloodstream of the patient. Within weeks, the patient began producing blood cells, indicating that the infused blood-forming stem cells, the "hematopoietic stem cells" (HSCs), had migrated to the marrow and, thereafter, had "engrafted" (i.e., proliferated within the marrow and had generated progeny cells in sufficient amounts to create all necessary blood elements). When I asked the patient's treating physician to explain how the cells home to the marrow, all he could say was that "the cells just know to go to the marrow". More importantly, he told me that very often (in ~25% of bone marrow transplants for aplastic anemia in that era), the HSCs failed to engraft and the patient would die within weeks of the transplant, in his opinion because the HSCs could not find their way to the marrow in the first place. It seemed impossibly paradoxical to clinical practice that a treatment intended to *cure* a patient could *hasten death* in as many as 25% of patients receiving the treatment. Thus began my interest in the molecular basis of cell migration, and, in particular, my pursuit of knowledge into how HSCs home to marrow. I wondered about the "homing receptor" that would guide marrow migration of HSCs: what is the structure of this molecule? How does it work? Also, most importantly, given its enormous potential to *cure* life-threatening blood diseases, I was both intellectually and emotionally drawn to HSC transplantation, and this is the area of medicine in which I have dedicated my entire clinical career.

In that same period of time, in the medical school classroom, I was learning about the pathobiology of infectious diseases. One particularly inspiring lecture highlighted the sentinel contributions of Robert Heinrich Herman Koch to bacterial culture techniques and to our understanding of the tubercle bacillus as the etiologic agent in tuberculosis. That lecture also described "Koch's postulates", a revolutionary advance in medical science in the late 1880s (whose origins could be traced to Jacob Henle, a mentor to Koch (Evans 1976)), whereby Koch established a standard for evidence in determining the causal relationship between a microbe and disease (Figure 1). But, for that era, well beyond its impact in microbiology, *Koch's postulates infused scientific rigor into medicine*, altering the cultural foundation of medical science from that of observation/association/correlation towards one grounded in *causal relationships yielding mechanistic insights*. Little did I know that the events of a HSC transplant and the lessons of the classroom would converge as I undertook research in the field of glycoscience.

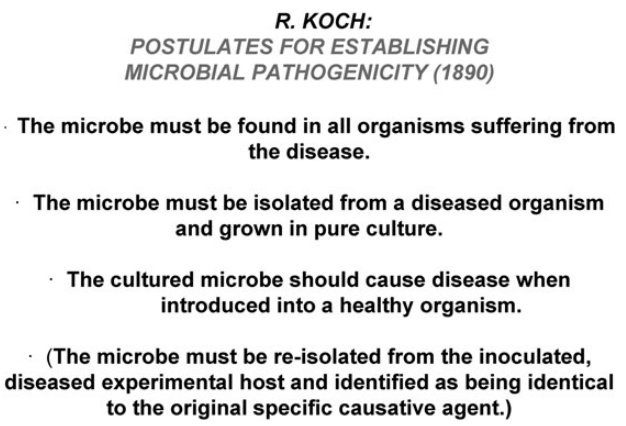

Fig. 1. Koch's postulates: Koch originally proposed only the first three criteria. Others have added the fourth condition (listed in parentheses), which, though a natural extension of Koch's ideas, was not formally stated nor emphasized by Koch.

The "homing receptor" concept

The "homing receptors"—molecules on the surface of blood-borne cells that guide organ/tissue migration—were first conceptualized over 50 years ago to explain observations regarding the extensive flux of lymphocytes between blood and lymph. In this process, known as "lymphocyte recirculation", lymphocytes emigrate from blood to lymph nodes, exit nodes via efferent lymphatics that subsequently coalesce into large lymphatic channels (e.g., the thoracic duct), which then drain into the vasculature from which the lymphocytes may then reenter lymph nodes. The strikingly nonrandom pattern of lymphocyte trafficking to lymphoid organs led to the notion that a "homing molecule" on the surface of lymphocytes directed trafficking to lymph nodes. This idea was supported by both physiologic and electron microscopic studies of James L.Gowans and colleagues (Gowans 1959; Gesner and Gowans 1962; Gowans and Knight 1964; Marchesi and Gowans 1964), providing evidence that lymphocyte migration to lymph nodes occurred via lymphocyte binding to defined microvascular structures consisting of plump, cuboidal endothelial cells known as "high endothelial venules" (HEV). Notably, electron microscopy studies of Marchesi and Gowans (1964) demonstrated lymphocyte adherence to HEV, and these investigations were prompted by earlier microscopic studies of leukocyte migration by Howard W. Florey (who shared the 1946 Nobel Prize for Medicine with Alexander Fleming and Boris Chain for discovery of penicillin and its clinical application). Florey was Gowans' post-doctoral mentor in the late 1940s, and his earlier studies showed that, in contrast to lymphocyte recruitment at HEV which occurred under steady-state conditions, leukocyte extravasation at sites of inflammation was a dynamic process. Florey noted that leukocyte diapedesis typically started within 2 hours of injury, occurring at post-capillary venules displaying adhesive properties (which he called a "sticky cement substance") that supported leukocyte binding and ensuing endothelial transmigration (Marchesi and Florey 1960; Florey and Grant 1961); interestingly, well before Florey's seminal findings, Julius Friedrich Cohnheim, an eminent German pathologist of the 19th century and early supporter of Koch's work (Sherrington 1910), performed real-time microscopy studies of microvessels in the frog and proposed that post-capillary venules at sites of inflammation underwent molecular alterations, a "change of a chemical character", that mediated attachment of flowing "colorless blood-corpuscles" (leukocytes) on the vessel surface and subsequent extravasation at sites of inflammation (Cohnheim 1889).

At the outset of observations on the specificity of leukocyte extravasation, there was no suggestion that carbohydrates were key components of this biophysical chemistry. The first studies to examine the biology of glycans in lymphocyte trafficking were performed in the mid-1960s by a former post-doctoral trainee of Gowans, Bertrand M. Gesner (Gesner and Ginsburg 1964). Later studies in the 1960s by Gesner with his (then) post-doctoral fellow, Judith J. Woodruff, showed that lymphocyte migration to lymph nodes was impaired following incubation of cells with trypsin, thus suggesting the existence of an HEV-seeking "homing" protein. The concept that a lymphocyte membrane protein mediates trafficking to lymph nodes was first proposed in the Discussion of Woodruff and Gesner's report: "The most apparent explanation is that trypsin cleaves constituents of the lymphocyte surface required for the selective emigration through the endothelial cells of the post-capillary venules in lymph nodes" (Woodruff and Gesner 1968).

The identification of this putative "lymph node homing receptor" was facilitated by an *in vitro* assay created by Woodruff and Hugh B. Stamper in the mid-1970s (the "Stamper–Woodruff assay") (Stamper and Woodruff 1976). This assay mimics physiologic binding of

lymphocytes to HEV, and consists of overlaying suspensions of viable lymphocytes onto glutaraldehyde-fixed thin (typically, <10 μm thickness) cryostat sections of lymph nodes, in the cold (4°–7°C) under shear conditions (as originally described, fluid shear delivered by a rotatory platform). In their landmark studies, these investigators correctly deduced that because lymphocyte-HEV adherence *in vivo* was occurring under hemodynamic flow conditions, the binding of lymphocytes to HEV *in vitro* would require shear stress. The fact that the assay was performed in the cold was fortuitous, as it avoided engagement of a variety of confounding adhesion molecules, particularly integrins, whose activity are blunted under sub-physiologic temperatures (Spertini et al. 1991). The Stamper–Woodruff assay allowed specific and reproducible analysis of the avid adhesion between lymphocytes and HEV, and in their initial description of assay results, the authors described the lymph node homing molecule as a lymphocyte surface "receptor" for HEV (Stamper and Woodruff 1976). This assay then enabled studies by Stephen D. Rosen and colleagues which revealed that the lymphocyte HEV receptor was a lectin and that sialylated glycans expressed on HEV served as the ligand for this lectin (Stoolman and Rosen 1983; Rosen et al. 1985; Rosen and Yednock 1986). The Stamper–Woodruff assay also facilitated the development of monoclonal antibody reagents that could neutralize the function of the receptor, initially described in the early 1980s by two investigators working separately, Yee Hon Chin, then a post-doctoral fellow working under Woodruff (Chin et al. 1983, 1984; Rasmussen et al. 1985), and by W. Michael Gallatin (Gallatin et al. 1983). The Chin mAb (known as A.11) was directed against the rat lymph node homing receptor, and the Gallatin mAb (known as MEL-14) was directed against the mouse homologue. Moreover, the Stamper–Woodruff assay also allowed for development of an mAb by Philip R. Streeter called "MECA79" that blocks the ability of HEV to support lymphocyte adherence (Streeter et al. 1988). The availability of the MECA79 mAb was critical to identifying a family of sulfated, sialofucosylated glycoproteins that serve as L-selectin ligands on HEV, collectively known as "peripheral lymph node addressins" (for review, see Rosen 2004).

Throughout most of the 1980s, the identity of the authentic lymph node homing receptor was unsettled due to various conflicting results. Some investigations suggested that a protein called the "Hermes" antigen served as the human lymph node homing receptor (Jalkanen, Bargatze, et al. 1986; Jalkanen, Reichert, et al. 1986; Jalkanen et al. 1987), and there were reports of immunologic cross-reactivity between Hermes and MEL-14 proteins (Jalkanen et al. 1987). Furthermore, immunoprecipitation studies using MEL-14 mAb initially indicated that the target antigen was ubiquitin (Gallatin et al. 1986; Siegelman et al. 1986; St John et al. 1986). In 1987, I joined the Chin lab as a post-doctoral fellow to investigate how lymphocytes homed to lymph nodes, and, specifically, to identify the "A.11" protein by expression cloning. Within 9 months of effort, by probing a λgt11 phage expression library of rat thoracic duct lymphocyte cDNA using a polyclonal antiserum raised against the A.11 protein, I deduced the coding sequence of A.11 (data reviewed in Chin et al. 1991). However, the confusion in molecular features of the lymph node homing receptor stymied the publication of this rat cDNA sequence. My efforts to clone the A.11 antigen (which turned out to be rat L-selectin) was my first brush with glycoscience; yet, at that time, I was more focused on elucidating the structural biology of L-selectin than that of its ligands. Nonetheless, in 1989, cloning of the lymph node homing receptor was reported by several groups (reviewed in Sackstein 1997), indicating that the molecule belonged to a group of lectins that require calcium for binding to their ligands (i.e., "C-type" lectins); other lectins with similar structural features (known at the time as ELAM-1 (now "E-selectin", CD62E) and GMP-140/PADGEM (now "P-selectin", CD62P)) were also cloned in 1989 (Bevilacqua et al. 1989; Johnston et al. 1989), and, collectively, these three proteins defined a family called "selectins" (reviewed in Bevilacqua et al. 1991; Bevilacqua and Nelson 1993). These cloning studies unequivocally separated the lymph node homing receptor, subsequently called "L-selectin" (CD62L), from the "Hermes" antigen, which was revealed to be CD44 (Goldstein et al. 1989; Picker et al. 1989). Notably, studies in the 1990s using the parallel plate flow chamber showed that L-selectin receptor/ligand interactions require a threshold level of fluid shear stress (Finger et al. 1996; Alon et al. 1997; Lawrence et al. 1997), supporting the underlying logic for choosing shear conditions to detect lymphocyte–HEV binding interactions in the Stamper–Woodruff assay. In contrast, CD44 ("Hermes") binding to its principal ligand, hyaluronic acid, occurs readily under non-shear (i.e., static) conditions.

Throughout the 1990s, data accumulated supporting a key role for L-selectin in directing lymphocyte migration to lymph nodes. However, a variety of cells that do not typically home to lymph nodes express L-selectin, including mature myeloid cells and hematopoietic progenitor cells (reviewed in Sackstein 1997), yet assays under fluid shear conditions showed that L-selectin expressed on granulocytes (a mature myeloid cell) was capable of binding to HEV ligands (Lawrence et al. 1995). These observations seemingly opposed the homing receptor concept: how could a homing receptor direct cell trafficking to a given target tissue for only a subset of cells that express that molecule? This mystery was resolved by the observation that a coordinated sequence of steps control physiologic migration of circulating cells into tissues. It thus became accepted canon that homing receptors are categorically required, but not sufficient by themselves, for tissue-specific migration.

Cell migration: the "multistep paradigm"

Studies throughout the 1980s and 1990s revealed that, in addition to homing receptors, cell migration is orchestrated by chemoattractants present within endothelial beds. The principal chemoattractants consist of a large family of small molecular weight glycoproteins known as chemokines, which characteristically engage G-protein-coupled receptors (GPCRs) expressed on circulating cells. Some chemokines are displayed in endothelial beds in a tissue-specific manner, and certain chemokines can be inducibly expressed at sites of inflammation. Data obtained from chemokine studies, combined with results of studies of homing receptors, yielded a model whereby cell migration is encoded by a series of overlapping steps (reviewed in Butcher 1991 and Springer 1994). According to this "multistep paradigm", the function of a homing receptor is not purely to direct cellular trafficking in a tissue-specific manner, but to function as a molecular brake in initiating binding to endothelial cells under hemodynamic shear stress (i.e., "Step 1" of the cascade of events culminating in extravasation). Specifically, a homing receptor exhibits the biophysical property of mediating initial decelerative tethering and then sustained rolling contacts of cells in blood flow onto the target tissue vascular endothelium at velocities below that of the prevailing hemodynamic stream (reviewed in Sackstein 2005). Engagement of chemokine receptors then results in "Step 2" intracellular signaling (i.e., "inside-out" signaling) that activates adhesiveness of cell surface integrins such as very late antigen-4 (VLA-4) and lymphocyte function-associated antigen-1 (LFA-1) to their respective endothelial ligands, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (Step 3), followed by endothelial transmigration (Step 4) (Springer 1994). Thus, tissue-specific migration is controlled by discrete combinations of homing receptors and chemokine receptors present on circulating

cells, recognizing a respective "traffic signal" consisting of relevant combinations of a homing receptor ligand together with chemokine (s) expressed within the target tissue microvascular endothelium in an organ-specific manner.

On circulating cells, expression of a homing receptor that engages the pertinent tissue endothelium is indispensable for extravasation, as tethering and rolling adhesive interactions are obligatory to allow recognition of chemoattractants and elaboration of other downstream events. But, should a cell lack the requisite chemokine receptor(s) for chemokine(s) present at the target endothelial bed, it may be incapable of undergoing integrin activation and thereby will not extravasate. As such, the discovery of chemokines solved the mystery of the specificity of lymphocyte homing to lymph nodes: HEV constitutively display chemokine CCL21 (SLC), and lymph node-homing lymphocytes express the receptor for this chemokine (known as "CCR7"). Granulocytes and HSC do not typically express CCR7, so, while these cells may undergo L-selectin-dependent rolling adhesive interactions on HEV, these cells do not typically migrate to lymph nodes.

Selectins and their ligands: principal effectors of Step 1

A variety of independent lines of evidence obtained throughout the 1990s and 2000s revealed that the most efficient effectors of Step 1 interactions are the selectins and their ligands. As noted above, the selectin family is comprised of three proteins, E-, P- and L-selectin (CD62E, CD62P and CD62L, respectively) (Bevilacqua and Nelson 1993), and (as nomenclature embedded in their name) these molecules are lectins that bind to specialized carbohydrate determinants, consisting of sialofucosylations containing an α(2,3)-linked sialic acid substitution(s) and an α(1,3)-linked fucose modification(s) prototypically displayed as the tetrasaccharide sialyl Lewis X (NeuAc-α(2,3)-Gal β(1,4)[Fuc-α(1,3)]-GlcNAc-β(1-R)) (Polley et al. 1991; Sackstein 2009). L-Selectin is expressed on circulating leukocytes and on hematopoietic progenitor cells, whereas E- and P-selectin are expressed on vascular endothelium, and P-selectin is also expressed on platelets (Sackstein 2005). In platelets and endothelial cells, P-selectin is stored in granules where it is rapidly translocated to the membrane in response to agonists such as thrombin. E-selectin is not stored in granules, and, notably, both E- and P-selectin are not typically expressed on endothelial cells: they are inducible endothelial membrane molecules that are characteristically expressed at sites of tissue injury and inflammation, prominently upregulated by inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF); in addition to inducing endothelial selectin expression, these cytokines also markedly upregulate expression of VCAM-1 (which, as noted above, is the ligand for the β1-integrin VLA-4). However, it is important to recognize that whereas IL-1 and TNF each induce transcription of mRNA encoding P- and E-selectin in rodents, the P-selectin promoter of primates lacks the relevant response elements for these cytokines and only E-selectin is induced by TNF and IL-1 in primates (Yao et al. 1999). This physiologic difference in regulation of P-selectin expression yields a key distinction in the biology of inflammation between rodents and humans: in rodents, both P- and E-selectin contribute to cell recruitment at inflammatory sites, but E-selectin dominates in humans.

Natively, most endothelial beds do not express vascular selectins. However, in mice, bone marrow and skin microvessels constitutively expresses both E- and P-selectin (Weninger et al. 2000; Sipkins et al. 2005), and constitutive E-selectin expression, but not P-selectin, is found in non-inflamed human bone marrow and skin microvessels (Schweitzer et al. 1996; Jung et al. 1997; Chong et al. 2004). Cell recruitment to skin and marrow in humans is dependent on E-selectin-mediated binding interactions more so than in mice, and intravital microscopy studies in transgenic mice bearing the human P-selectin gene have shown that E-selectin dominates basal rolling interactions in the skin of the transgenic mice (analogously to humans), whereas P-selectin does so in wild-type animals (Liu et al. 2010). Thus, steady-state homing of circulating cells to human skin and bone marrow in humans depends on expression of E-selectin ligands that serve as "skin homing receptors" and "bone marrow homing receptors", respectively.

In humans, lymphocyte migration to skin depends on expression of a structure known as "cutaneous lymphocyte antigen" (CLA). This determinant was historically defined by its reactivity to a rat IgM mAb known as HECA-452 (Duijvestijn et al. 1988) that recognizes sialofucosylated structures such as sLe^x (and its isomer sLe^a) (Berg, Robinson, et al. 1991). Early immunohistochemistry studies revealed that most lymphocytes resident in human skin are reactive with HECA-452 (thus, are CLA+) (Duijvestijn et al. 1988). Subsequent biochemical studies showed that CLA serves as a ligand for E-selectin (Berg, Yoshino, et al. 1991), and that CLA is a specialized glycoform of a protein known as "P-selectin glycoprotein ligand-1" (PSGL-1; CD162), a leukocyte molecule that serves as the principal ligand for P-selectin and that also binds L-selectin (Fuhlbrigge et al. 1997). Thus, the term "CLA" refers broadly to expression of HECA-452-reactive glycans (e.g., sLe^x), but in its strictest sense "CLA" defines a PSGL-1 glycoform that is reactive with mAb HECA-452. In fact, on various leukocytes, HECA-452-reactive glycans may be displayed on non-PSGL-1 scaffolds that can serve as ligands for E-selectin and may thereby promote migration to skin, including the protein known as "leukosialin" (CD43) (Fuhlbrigge et al. 2006) and glycolipids (Nimrichter et al. 2008). With the discovery that display of cell surface sLe^x (as identified by HECA-452 mAb) was key to cellular trafficking to skin, lymphocyte trafficking to lymph node and lymphocyte trafficking to skin were thus operationally linked as functions of selectins: L-selectin mediates homing of lymphocytes to lymph node by binding to ligands bearing relevant sialofucosylated structures on HEV recognized by mAb MECA79, whereas CLA and other HECA-452-reactive structures on relevant lymphocytes (e.g., skin-homing effector memory cells) engages E-selectin that is permanently expressed on dermal microvasculature.

Searching for the bone marrow homing receptor: my induction into the field of glycoscience

As mentioned in *Introduction*, my interest in the molecular basis of HSC migration to marrow steered my career into glycoscience. Flow cytometry studies of normal human marrow cells undertaken in my lab and in the labs of other investigators in the late 1980s and early 1990s showed that L-selectin is expressed on nucleated marrow cells in a highly regulated, bimodal manner (Kansas and Dailey 1989; Terstappen et al. 1992). L-Selectin is not expressed on marrow erythroid and megakaryocytic progenitors nor on cells in intermediate stages of leukocyte development (both lymphoid and myeloid progenitors) (reviewed in Sackstein 1997). However, it is typically present on mature marrow myeloid and lymphoid cells and on cells identified as hematopoietic stem/progenitor cells ("HSPCs", defined as cells expressing a glycoprotein called "CD34" that is present only on early hematopoietic progenitors, but, notably, is also present on endothelial cells; "early" HSPCs are identified by expression of CD34 in the absence of markers associated with blood cell lineage commitment, i.e.,

CD34+/lin- cells). The expression of L-selectin on human HSPCs immediately caught my attention: why would a membrane molecule best known for serving as the "lymph node homing receptor" be expressed on HSPCs when these cells do not function as immunologic effectors and are, for the most part, anatomically confined to the marrow in humans?

From an evolutionary perspective, it seemed odd to me that metabolic energy would be wasted in expressing L-selectin among human HSPCs if there were no function for this molecule within marrow microenvironments that regulate hematopoietic events. Based on this premise, I hypothesized in 1990 that L-selectin receptor/ligand adhesive interactions would contribute to homing and/or lodgment of the HSPCs within marrow microenvironmental "niches" critical for hematopoietic cell proliferation and differentiation. This hypothesis gained some support by studies published in 1992 showing that incubation of human HSPCs *in vitro* with anti-L-selectin antibody blocked clonogenic outgrowth of cells in both long-term (stromal) and short-term (methylcellulose) assays (Gunji et al. 1992). Notably, studies in mice in the late 1980s had suggested a role for cell surface lectins in homing to marrow (Aizawa and Tavassoli 1987; Hardy and Tavassoli 1988), and, in the early 1990s, it was also reported that a glycoform of CD34 expressed on murine lymph node HEV was reactive with L-selectin and with the MECA79 antibody (Imai et al. 1991; Baumheter et al. 1993). Though studies indicating that CD34 on HEV served as an L-selectin ligand had been performed in mice and solely on CD34 isolated from lymph node HEV, these results led to speculation that CD34 expressed on *human HSPCs* functions as a ligand for L-selectin, and that this L-selectin/CD34 receptor/ligand interaction promotes HSPC homing to, and lodgment in, marrow, respectively. To directly assess whether HSPC CD34 could serve as an L-selectin ligand, my lab undertook Stamper–Woodruff assays of native hematopoietic cells from human marrow and of cells from various human hematopoietic cell lines (Oxley and Sackstein 1994). We observed that the human CD34+ leukemic cell line KG1a and a major subset of native marrow-derived human CD34+ cells supported L-selectin-dependent lymphocyte adherence, providing first evidence of the existence of an L-selectin ligand on a non-endothelial cell type. However, a variety of different experimental approaches in my lab provided strong evidence that CD34 itself was not the ligand (Oxley and Sackstein 1994).

Subsequent to my lab's initial description of L-selectin ligand activity of human HSPCs, other investigators found that the leukocyte molecule PSGL-1 is also expressed on human HSPCs and binds to both P- and L-selectin (binding to each dictated within an overlapping NH_2-terminal binding site requiring O-linked sialofucosylations and tyrosine sulfation) (for review, see Carlow et al. 2009). However, our biochemical studies in the mid-1990s clearly distinguished the HSPC L-selectin ligand detected by Stamper–Woodruff assay from all other previously identified L-selectin ligands (including PSGL-1 and HEV L-selectin ligands): the human HSPC ligand possessed sulfation-independent binding activity, the ligand was not reactive with MECA79 mAb, and, most notably, rather than expressing the relevant sialofucosylated L-selectin binding determinants on *O*-glycans (as is characteristic of all known L-selectin ligands of that era), the HSPC ligand displayed its L-selectin binding determinants on N-linked glycans (Sackstein et al. 1997; Sackstein and Dimitroff 2000). Importantly, though we consistently observed PSGL-1 expression on native human HSPCs, this molecule could not engage L-selectin under the shear stress conditions imposed by the Stamper–Woodruff assay (Sackstein and Dimitroff 2000), underscoring the uniquely robust L-selectin binding activity of the HSPC L-selectin ligand.

Our efforts to identify the HSPC L-selectin ligand were extremely challenging and frustrating. Engagement of L-selectin to its ligands requires hemodynamic shear, and parallel plate flow chamber assays showed that L-selectin binding to the HSPC L-selectin ligand required a minimum of 1.0 dyne/cm^2 shear stress. Our attempts to immunoprecipitate the ligand with L-selectin-immunoglobulin chimeric construct (L-selectin-Ig) consistently failed due to our inability to recapitulate the proper adhesive shear stress level under aqueous conditions. Moreover, though our biochemical studies indicated that sLe^x served as the L-selectin binding determinant of the HSPC L-selectin ligand, incubation of cells with combinations of anti-sLe^x mAb (including mAb HECA-452) did not blunt L-selectin-dependent binding in Stamper–Woodruff assays, and imunoprecipitation of lysates of native human HSPCs and KG1a cells with anti-sLe^x mAbs yielded a complex assortment of proteins (including PSGL-1). However, in using *N*-glycanase to analyze the relevant carbohydrate linkage of L-selectin binding determinants, a critical clue materialized: L-selectin binding activity of the human HSPC L-selectin ligand was unaffected by sodium dodecyl sulfate (SDS) and β-mercaptoethanol denaturing conditions used routinely in the *N*-glycanase buffer (Sackstein and Dimitroff 2000). I surmised, therefore, that the ligand's binding activity would withstand SDS–polyacrylamide gel electrophoresis (SDS–PAGE) conditions and the subsequent transfer of protein onto PVDF membranes. Accordingly, we performed SDS–PAGE of KG1a membrane preparations, blotted the gel onto PVDF membranes, then placed the membrane into the parallel plate flow chamber wherein L-selectin+ lymphocytes were infused under fluid shear conditions. We named this novel technology the "blot rolling assay" (for technical details, see (Sackstein and Fuhlbrigge 2009)), and it revealed highly avid L-selectin-dependent lymphocyte rolling on an ~90 kDa band (Dimitroff et al. 2000). We excised that band, and mass spectrometry revealed that the protein consisted of a novel sialofucosylated glycoform of CD44 (Dimitroff et al. 2000). Blot rolling assays were then undertaken using infused Chinese hamster ovary (CHO) cells transduced to express either E-selectin (CHO-E cells) or P-selectin (CHO-P cells). These studies showed that the ~90 kDa band strongly supported E-selectin binding, but not P-selectin binding (Dimitroff, Lee, Rafii, et al. 2001), and additional biochemical and functional studies showed that this CD44 glycoform is the most potent E- and L-selectin ligand expressed natively on human cells (Dimitroff, Lee, Rafii, et al. 2001; Dimitroff, Lee, Schor, et al. 2001). Owing to the fact that the molecule was first identified on native human HSPCs, I named it "hematopoietic cell E-/L-selectin ligand" (HCELL).

Though HCELL expression clearly depends on CD44 expression, it is imprecise and misleading to state that "CD44 is a selectin ligand". The CD44 protein sequence is extremely polymorphic, with numerous isoforms generated by alternative splicing, yielding remarkable functional pleiotropism that has led to distinct nomenclature for certain CD44 isoforms with unique properties (for review, see Sackstein 2011). On human HSPCs, HCELL is expressed mainly on the "standard" CD44 isoform (called "CD44s") lacking any peptide products of splice sequences. CD44 is best known for being the principal receptor for hyaluronic acid, and prior to discovery of HCELL, it was believed to function solely in cell–matrix adhesive interactions, not in cell–cell adhesive interactions. CD44 is a lectin (i.e., it binds the glycosaminoglycan hyaluronic acid), whereas HCELL is a lectin ligand, and, in particular, the selectin binding activity of HCELL is not mediated by the CD44 protein, it is governed by sLe^x determinants that are displayed on N-linked glycans that decorate the CD44 scaffold. As described in the paragraph above, we consistently found that selectin binding by HCELL is maintained under SDS–PAGE and other protein

denaturing conditions, and, notably, both E- and L-selectin binding of HCELL are unaffected by the fixative glutaraldehyde, which markedly alters protein conformation but does not affect glycan structure (Oxley and Sackstein 1994). Furthermore, our early studies showed that inhibition of N-glycosylation, that enzymatic removal of *N*-glycans, and that α(2,3)-sialidase or α(1,3)-fucosidase digestion in each case eliminated ligand activity, highlighting the functional dependency on glycans, specifically, on N-linked terminal sialofucosylations displayed as sLe^x motifs, on the CD44 scaffold (Oxley and Sackstein 1994; Sackstein and Dimitroff 2000; Dimitroff, Lee, Rafii, et al. 2001). Collectively, these results indicate that the CD44 protein is essentially inert with regard to HCELL activity, i.e., *the carbohydrate modifications are the "working end" of HCELL*, and this key fact sealed my fascination with the field of glycoscience.

The multistep model of HSPC homing to bone marrow

The molecular basis of HSPC homing to marrow was elucidated within the 1990s and early 2000s on information derived predominantly from mouse models, but also including studies utilizing xenogeneic transplants of human HSPC in immunocompromised mice (reviewed in Sackstein 2004). Abundant data were obtained indicating that HSPC surface expression of E-selectin ligand(s), of the chemokine receptor CXCR4, and of the β1-integrin VLA-4 each contributed to HSPC homing to marrow (see Figure 2). Mouse intravital microscopy studies revealed that HSPC migration to marrow occurs at specific microvascular endothelial beds (sinusoids) that constitutively express E-selectin, that these E-selectin-bearing sinusoidal vessels have a highly restricted distribution, and that expression of the chemokine CXCL12 (otherwise known as "SDF-1"; i.e., the ligand for CXCR4) co-localizes precisely with expression of E-selectin (Sipkins et al. 2005). Thus, the conjoint expression of E-selectin and CXCL12 within specialized sinusoidal vessels creates a hotspot for HSPC recruitment to marrow. Furthermore, intravital microscopy revealed that the ligand for VLA-4, VCAM-1, is also constitutively expressed on marrow microvessels in a more general pattern to that of E-selectin, but which overlaps the expression of E-selectin (Sipkins et al. 2005). Collectively, these findings elucidated the principal components of the multistep process of HSPC homing to marrow: E-selectin receptor/ligand interactions mediate Step 1, allowing engagement of HSPC CXCR4 to chemokine CXCL12 resulting in activation of VLA-4 (Step 2), with ensuing VLA-4 firm adherence on VCAM-1 (Step 3) and transmigration (Step 4) (Figure 2).

Glycoscience and Koch's postulates: defining the role of HCELL in HSPC homing to marrow

The predominant role of E-selectin in governing Step 1 adhesive interactions in marrow sinusoidal vessels, together with the characteristic expression of HCELL on HSPCs and the extremely high avidity of HCELL for E-selectin, led me to reason that HCELL could serve as the principal human "bone marrow homing receptor". With reference to Koch's postulates, I elaborated a set of guiding principles to ascertain the causal relationship between expression of a given glycoconjugate and an observed biologic property, in this case, to establish whether HCELL served as an authentic HSPC bone marrow homing receptor (Figure 3). As listed in Figure 3, the First Postulate was satisfied by the strict correlation of human HSPC expression of HCELL; the Second Postulate was satisfied by the demonstration that CD44 immunoprecipitated from KG1a cells and from human HSPCs had robust E-selectin-binding activity (i.e., the bioactivity requisite for the biologic property). However, neither of these findings provided direct evidence that expression of HCELL itself endows homing of cells to marrow (the biologic property). To address the Third Postulate, I sought to develop a method to custom-modify CD44 glycans on the cell surface to engender HCELL expression. To this end, a platform technology called "glycosyltransferase-programmed stereosubstitution" (GPS) was created, with specific intent to glycan engineer the surface of living cells using glycosyltransferases and necessary reagents/enzymatic conditions in a form that would not affect cell viability or native cell phenotype (reviewed in Sackstein 2009; technical details are described in Sackstein 2010). To assess the distinct contribution of HCELL to marrow homing, I desired to glycoengineer HCELL expression on a cell that expresses CD44 but, ideally, is otherwise devoid of all E-selectin ligands, and thereby directly evaluate whether enforced HCELL expression would steer the cell to the marrow. Of various cells tested, I settled on use of human mesenchymal

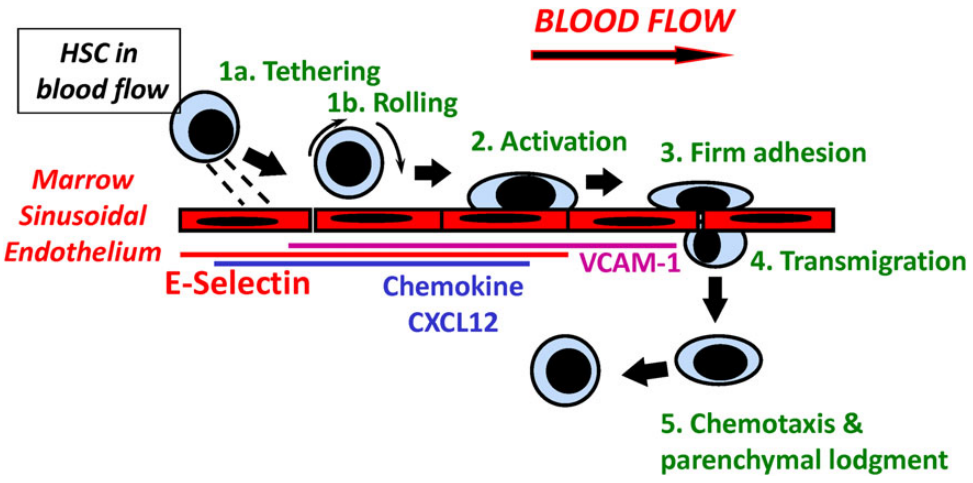

Fig. 2. The multi-step model of human HSC migration to marrow. Schematic representation of the multiple steps involved in human HSC migration from the vasculature into bone marrow. HSC express E-selectin ligands, CXCR4 (the receptor for chemokine CXCL12) and VLA-4 (a β1-integrin that binds to VCAM-1). Recruitment of HSC to marrow occurs at specialized marrow sinusoidal endothelial beds constitutively bearing co-localized expression of E-selectin, chemokine CXCL12 (SDF-1) and VCAM-1.

stem cells (MSCs), as these cells natively lack E-selectin ligands and express copious amounts of CD44. MSCs are also precursors of osteoblasts; as such, it would be possible to analyze whether homing of human MSCs into mouse marrow would engender production of human osteoid within mouse bone.

Though MSC comprise a very minor population of cells in nearly all tissues, they can be readily expanded in culture from various primary tissue sources. Bone marrow is a well-recognized source of MSCs, and, based on the presumption that MSC from bone (as opposed to MSC from other sources, e.g., adipose tissue) might have a particular proclivity to create osteoblasts, MSCs were culture-expanded from normal marrow obtained from donors for HSC transplantation. The marrow-derived human MSC characteristically expressed VLA-4 but not CXCR4, and biochemical studies showed that the cells exhibited high levels of a sialylated glycoform of CD44, containing asparagine-linked (i.e., N-linked) glycans with terminal α(2,3)-sialyllactosamines that were lacking only α(1,3)-fucosylation at *N*-acetylglucosamine to realize the complete sLe^x determinant (Sackstein et al. 2008) (see Figure 4). Accordingly, the native surface CD44 of human MSC was converted into HCELL by exofucosylation using an α(1,3)-linkage-specific fucosyltransferase, fucosyltransferase VI (FTVI), together with its relevant donor nucleotide sugar, GDP-fucose (Figure 4). This FTVI enzyme was expressly formulated (together with attendant reactants and reaction conditions) for high-efficiency enzyme activity without affecting MSC viability or differentiation to typical progeny, including osteoblasts.

ORGANIZING PRINCIPLES OF TRANSLATIONAL GLYCOBIOLOGY:
POSTULATES FOR ESTABLISHING GLYCONJUGATE BIOACTIVITY

- **The glycoconjugate must be present among cells/organisms expressing the relevant biologic property.**
- **The glycoconjugate should display the relevant bioactivity that is requisite for the biologic property when it is purified from the host cell/tissue.**
- **Enforced expression of the glycoconjugate on a cell/organism that does not natively express the glycoconjugate should confer the relevant biologic property.**
- **Following enforced expression of the relevant glycoconjugate on a target cell/organism which does not natively express the molecule, the isolated glycoconjugate should display the relevant bioactivity and structural features homologous to that of the isolated native molecule.**

Fig. 3. Postulates for establishing glycoconjugate bioactivity. Koch's postulates modified in relation to glycoscience, providing benchmarks for defining the bioactivity of a given glycoconjugate. See text for details on application of these postulates in validating the identity and function of HCELL as an authentic "bone marrow homing receptor."

Protease digestion studies following FTVI treatment of human MSCs showed that sLe^x determinants were created almost exclusively on glycoproteins (i.e., there was essentially no contribution of glycolipids to sLe^x display), and western blot staining using HECA-452 mAb revealed that the principal glycoprotein target of surface α(1,3)-fucosylation was CD44 (i.e., the CD44 protein was the predominant carrier of sLe^x) (Sackstein et al. 2008). Parallel plate flow chamber studies on HUVEC monolayers stimulated by TNF to express E-selectin showed that whereas buffer-treated (i.e., HCELL-) human MSC did not interact with the stimulated endothelial cells, exofucosylated (i.e., HCELL+) MSC exhibited potent E-selectin-dependent binding interactions under hydrodynamic shear conditions. These findings indicated that the glycoengineered HCELL was operationally similar to the molecule as natively expressed on human HSPCs. Indeed, we observed that glycoengineered HCELL on human MSCs was capable of engaging E-selectin at shear stress levels of upwards of 30 dyns/cm^2, thereby endowing E-selectin-binding activity equivalent to that of human HSPCs. But, most importantly, following intravenous infusion of HCELL$^+$ human MSC into immunodeficient mouse hosts, intravital microscopy of the calvarium showed robust MSC tethering and rolling interactions on mouse marrow sinusoidal vessels, with evidence of parenchymal infiltrates (i.e., extravasated

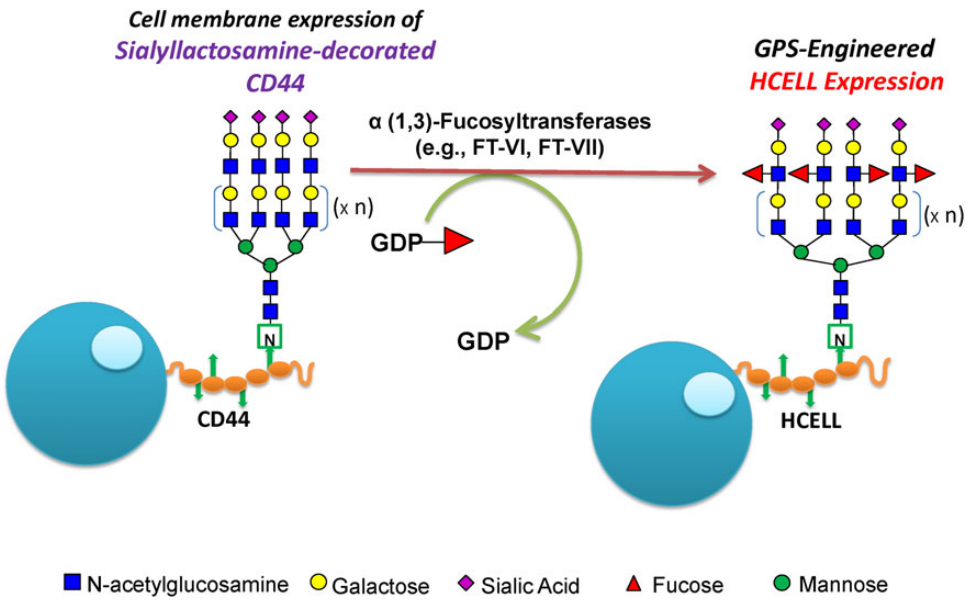

Fig. 4. Exofucosylation of sialylated CD44 to create HCELL via glycosyltransferase-programmed stereosubstitution (GPS). CD44 displaying a tetra-antennary N-linked glycan is shown, with polylactosamine backbone depicted by brackets and "x n". Fucosyltransferase-mediated α(1,3)-specific fucosylation of *N*-acetylglucosamine within the terminal α(2,3)-sialylated "Type 2" lactosamine unit (NeuAc-α(2,3)-Gal-β(1,4)-GlcNAc-β(1-R)) of CD44 yields HCELL.

MSC) within 1 hour post-infusion. In contrast, HCELL$^-$ MSC displayed modest interactions with marrow sinusoidal vessels, without significant marrow infiltrates even at 48 hours post-infusion. These findings satisfied the Third Postulate. The Fourth (and final) Postulate (see Figure 3) was fulfilled by biochemical studies showing that HCELL isolated from the glycoengineered MSCs binds to E-selectin and that the relevant sLe^x binding determinants are expressed on *N*-glycans, just as in HCELL natively expressed on human HSPCs.

Apart from homing to marrow, we observed that HCELL$^+$ MSC retained their native cell differentiation phenotype within the marrow *in vivo*. Within hours of systemic injection, extravasated HCELL$^+$ MSC lodged within marrow endosteal surfaces, and weeks later created human osteoid in mouse marrow (Sackstein et al. 2008), a finding that was particularly striking in that the host immunodeficient mice had normal skeletal architecture (i.e., had no skeletal diseases/deficits that would favor engraftment of human cells). Thus, enforced HCELL expression programmed osteotropism of human MSCs, yielding functional human osteoblast progeny under highly stringent competitive conditions for xenoengraftment.

To further assess the function of HCELL in piloting tropism of intravascularly administered cells to bone marrow (Figure 3, Postulate 3), we have performed complementary studies of enforced HCELL expression on mouse HSPCs. Unlike human HSPCs, mouse HSPCs do not express HCELL, but treatment of these cells with FTVI creates HCELL (Merzaban et al. 2011). Notably, mouse HSPCs natively display two glycoprotein E-selectin ligands, CLA and CD43-E (a CD43 glycoform that binds E-selectin), but exofucosylation does not increase expression of these structures (i.e., neither CLA nor CD43 are targets of exofucosylation). Enforced HCELL expression of mouse HSPCs profoundly increases E-selectin ligand activity (>4-fold), and HCELL$^+$ mouse HSPCs display >3-fold more marrow migration than do HCELL$^-$ HSPCs (Merzaban et al. 2011). Collectively, the data from human MSC and mouse HSPC studies provide strong physiologic evidence that HCELL serves as a genuine "bone marrow homing receptor".

HCELL as a "homing receptor": revisiting an historical concept

One remarkable finding of our *in vivo* studies of intravenously administered human HCELL+ MSC into mice was these MSCs readily extravasated at marrow microvessels in the absence of CXCR4 expression by the cells. This observation prompted us to examine whether transendothelial migration of human MSC might be mediated by chemokine-independent molecular effectors. Under both static and hydrodynamic flow conditions, we found that human MSCs exhibit transendothelial migration in the absence of exogenous chemokine input on TNF-stimulated human endothelial monolayers expressing both E-selectin and VCAM-1 (Thankamony and Sackstein 2011). Moreover, we observed that engagement of human MSC CD44 with hyaluronic acid, or of (glycoengineered) HCELL with E-selectin, in each case triggers a Rac1/Rap1-GTPase signaling pathway that induces VLA-4 firm adhesion to its ligands VCAM-1 and fibronectin. Consistent with these findings, our biochemical studies revealed that engagement of either CD44 or HCELL with their ligands stimulates physical co-association of CD44/HCELL with VLA-4, forming a bimolecular complex, and that subsequent VLA-4 activation is dependent on G-protein-coupled signaling mediated by ligation of CD44/HCELL (Thankamony and Sackstein 2011). This "Step 2-Chemokine By-pass Pathway" is driven by mechanosignaling induced by CD44 ligation, triggering "inside-out" VLA-4 integrin activation without chemokine-mediated stimulation (Figure 5). This coordinated cross-talk between a Step 1 effector (CD44/HCELL) with a Step 3 effector (VLA-4) harkens back to earlier concept(s) regarding the sufficiency of homing receptors in programming tissue migration: in some cases, *engagement of a homing receptor may be enough to not only achieve the needed initial shear-resistant adhesive interactions on target tissue endothelial beds, but may also directly license integrin adhesiveness, firm adhesion, and transendothelial migration.*

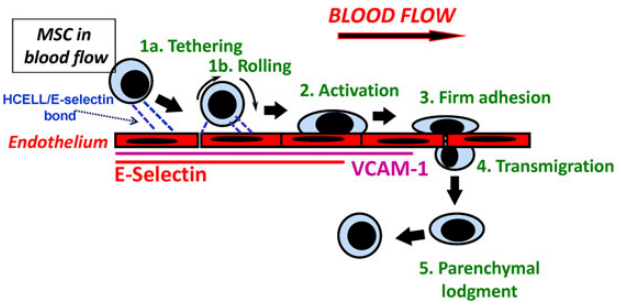

Fig. 5. The Step 2 chemokine-bypass pathway of homing. Schematic representation of MSC–endothelial interactions mediated by HCELL/E-selectin and VLA-4/VCAM-1 adhesive interactions. Human MSCs in circulation that express both HCELL and VLA-4 will extravasate, in the absence of stimulation by chemokines and other chemoattractants, at endothelial beds that co-express E-selectin and VCAM-1 (e.g., at marrow sinusoidal microvessels and, more generally, at TNF/IL-1—stimulated post-capillary venules).

Beyond the marrow: role of HCELL in programming cell migration to sites of inflammation

The fact that engagement of CD44/HCELL triggers upregulation of VLA-4 adhesiveness, and that inflammatory cytokines upregulate expression of E-selectin and VCAM-1 at all sites of tissue injury (Yusuf-Makagiansar et al. 2002; Sackstein 2005), raised the hypothesis that enforced HCELL expression could program extravasation of systemically-delivered VLA-4$^+$ cells to all sites of tissue injury regardless of chemokine receptor expression on the cells. As noted above, CD44 is widely expressed on many cells types, and VLA-4 is characteristically expressed on adult stem cells derived from marrow (i.e., HSCs and MSCs) and from tissue sources (e.g., neural stem cells (Pluchino et al. 2005)), and is also expressed on lymphocytes (Sackstein 2005). Accordingly, glycoengineering of cell surface CD44 to enforce HCELL expression could enable all forms of adoptive cell therapeutics, from stem cell-based regenerative therapeutics to cell-based immunotherapy (e.g., for tissue delivery of cytotoxic T cells or regulatory T cells). To address this possibility, we have investigated the role of HCELL in directing stem cell trafficking to inflammatory sites in mouse models of two prevalent autoimmune diseases: diabetes (using model of non-obese diabetic mice (NOD)) and multiple sclerosis (using model of experimental autoimmune encephalomyelitis (EAE)) (Abdi et al. 2015; Merzaban et al. 2015). In each case, glycoengineering of HCELL expression on the relevant murine stem cells (MSC in diabetes study; neural stem cells in EAE study) resulted in enhanced trafficking of the cells to inflammatory sites (pancreatic islets in diabetes; central nervous system plaques in EAE) with profound biologic effects (durable reversal of hyperglycemia in NOD model; amelioration of neural deficits in EAE model) (Abdi et al. 2015; Merzaban et al. 2015). Notably, in the NOD model, we observed that

despite expression of robust surface sLe^x determinants on FTVI-treated CD44$^{-/-}$ MSC, these CD44$^-$ MSC lack the capacity to colonize pancreatic islets in diabetic mice and did not induce durable reversal of hyperglycemia. These findings indicate that E-selectin binding determinants displayed on the CD44 scaffold are required to achieve the observed biologic effects, and highlight the key role of the CD44/VLA-4 bimolecular complex in driving extravasation at inflammatory sites.

Conclusion

This review has described a career journey that was inspired by a clinical puzzle (*what is the "bone marrow homing receptor" that pilots HSPC migration to marrow?*), herein detailing the then-available knowledge at each time point during the ensuing trek within the terrain of glycoscience. In the greater context of translational glycobiology, what began as a rather focused question yielded discovery of a novel glycoform of CD44, HCELL, and has evolved into a compelling strategy for custom-modifying cell surface glycans on CD44 to enable adoptive cell therapies for a wide variety of clinical indications. During this research odyssey, I framed a set of organizing principles for translational glycobiology: postulates for establishing glycoconjugate bioactivity were set forth that were motivated by Koch's postulates, and these postulates navigated the effort in ascertaining the causal relationship between expression of HCELL and cell migration to marrow.

It is now clear that glycoengineered HCELL, on a variety of cell types, displays structural and biologic fidelity with that of the native molecule expressed on human HSPCs. The HCELL molecule is a highly potent E-selectin ligand, and is a major effector of cell migration to any site where E-selectin is expressed. For migration to marrow, HCELL meets all the operational criteria to be considered a genuine "homing receptor": it is expressed natively on relevant cells that home to marrow (HSPCs), it binds robustly to its cognate ligand (E-selectin) that is constitutively expressed on target tissue endothelium (i.e., marrow sinusoidal vessels), and enforced expression endows cells with the ability to travel to the intended anatomic site (i.e., MSC migration to marrow). The original homing receptor hypothesis proposed that a distinct molecule on the surface of a cell would function to direct migration to a given target tissue, and the fact that engagement of HCELL can trigger integrin adhesiveness and transmigration in the absence of chemokines validates the fundamental concept that a homing receptor can itself create the "address" for cell delivery. In clinical context, our studies to date indicate that glycoengineering of cell surfaces to engender HCELL expression licenses cell migration not only to marrow but also to inflammatory sites. For adoptive cell therapies, primary clinical principles dictate that the vascular route of cell administration is preferred as it allows for ease in repeated administration of therapeutic cells, avoids the potential of tissue/organ damage by direct tissue injection, and permits appropriate cell delivery for systemic diseases (e.g., osteoporosis) or for tissues with anatomy unfavorable to direct injection (e.g., lung, pancreas, CNS, etc.). Thus, translational glycobiology has made possible the steering mechanism to drive site-specific delivery of intravascularly administered cells, literally opening the "avenue" for fulfilling the enormous promise of cellular therapeutics and thereby improving patient outcomes for a wide variety of disabling and life-threatening conditions.

Acknowledgements

This review summarizes over 25 years of effort to uncover the mysteries of cell trafficking, an effort truly inspired by my patients undergoing HSC transplantation. Thus, I thank my patients, alive and deceased, for their lessons of humanity and courage, and I am optimistic that the efforts in translational glycobiology described here will yield the intended benefit(s) to their care and welfare. I am eternally indebted and grateful to all my mentors (in particular, E. Donnall Thomas, Konrad E. Bloch, Yee Hon Chin, J. Wayne Streilein, Harvey R. Colten, Edgar Haber, Ramzi S. Cotran, Robert A. Good, Edward O. Wilson, Murray Epstein and Adel A. Yunis) for instructing me on how to observe nature, for rigorously training me in the scientific method, for educating me on how to apply experimental controls consistently, and, by their actions and deeds, for enlightening me to think boldly and think critically. Finally, but certainly not least, I express my deepest thanks to all my talented and devoted co-workers (including numerous colleagues, technicians, students and post-doctoral fellows) for their invaluable assistance in elucidating the structure and biology of HCELL and for help in the development of GPS. I am extremely grateful for the support of this work over the past three decades by the Department of Veterans Affairs (Research Career Development Award) and by the National Institutes of Health (NHLBI grants PO1 HL107146, RO1 HL60528 and RO1 HL73714, and NCI grant RO1 CA121335), and, in particular, for the efforts of Dr. Rita Sarkar at the National Heart Lung Blood Institute for launching the Programs of Excellence in Glycosciences, an initiative designed by Dr. Sarkar specifically to accelerate the pace of translational glycobiology.

Conflict of interest statement

According to National Institutes of Health policies and procedures, the Brigham & Women's Hospital has assigned intellectual property rights regarding HCELL and GPS to the inventor (the author). The author's ownership interests were reviewed and are managed by the Brigham & Women's Hospital and Partners HealthCare in accordance with their conflict of interest policy. The author is a founder of Warrior Therapeutics LLC, and serves as a consultant to DaVinci Biosciences and to Mesoblast, LTD. Portions of the GPS technology have been licensed to Warrior Therapeutics LLC, to Mesoblast LTD and to Bio-techne, Inc.

Abbreviations

CHO, Chinese hamster ovary; CLA, cutaneous lymphocyte antigen; EAE, experimental autoimmune encephalomyelitis; FTVI, fucosyltransferase VI; GPCRs, G-protein-coupled receptors; GPS, glycosyltransferase-programmed stereosubstitution; HCELL, hematopoietic cell E-/L-selectin ligand; HEV, high endothelial venules; HSCs, hematopoietic stem cells; ICAM-1, intercellular adhesion molecule-1; IL-1, interleukin-1; LFA-1, lymphocyte function-associated antigen-1; MSCs, mesenchymal stem cells; NOD, non-obese diabetic mice; SDS, sodium dodecyl sulfate; TNF, tumor necrosis factor; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

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