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COMMENT

Positioning the immune system: unexpected roles for α6-integrins

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Accurate initiation and regulation of immune responses requires the dynamic recruitment and retention of lymphocytes in lymphoid tissue. It is now well accepted that the cells of the innate and adaptive immune systems reposition themselves in response to changes in the pattern of expression of chemokines and chemokine receptors. In fact, chemokines drive not only the movement of cells between and within lymphoid microenvironments, but also modify the affinity of integrins to ensure that cells recruited to a particular location remain there. The last few years have seen great advances in our understanding of the roles of individual chemokines and their receptors in regulating movement of lymphocytes in the bone marrow and peripheral lymphoid tissues and, indeed, in lymphoid organogenesis. Similar progress has been made in appreciation of the structural biology of integrins, such that mechanisms of ligand binding (the structural consequences of integrin activation) are becoming much better understood. The work of Helen Ambrose and Simon Wagner, published in this issue of *Immunology*[1] demonstrates a striking up-regulation of α6-integrin expression by murine germinal centre (GC) B cells, suggesting an important role for α6-containing integrins in the GC.

BASIC BIOCHEMISTRY OF CHEMOKINES AND INTEGRINS

Chemokines are proteins that function as chemoattractants, inducing cellular chemotaxis along a concentration gradient towards the chemokine.[2] Chemokines act via receptors that have seven transmembrane loops and are coupled to intracellular signalling systems via heterotrimeric G proteins. Chemokine receptor signalling also regulates cellular responses such as transcription (of cytokine genes), respiratory burst and, importantly, modulation of integrin affinity.

An integrin comprises α and β transmembrane glycoprotein subunits in a non-covalent complex.[3] There are 18 α subunits and eight β subunits, and 24 heterodimeric combinations are currently recognized. Alternative splicing of transcripts of certain integrin subunit genes (including the α6-integrin)[4] increases the number of possible heterodimers. The main target motif for binding of integrins to matrix ligands, such as fibronectin, vitronectin and laminins, is the Arg-Gly-Asp (RGD) tripeptide, although other binding sites have been described.[5] Integrins can transmit signals to the cell interior upon ligand binding (so-called 'outside-in' signalling) and are themselves targets of signalling pathways. Thus, reversible phosphorylation of the cytoplasmic subunits strikingly alters the affinity of the integrin for ligand; this is 'inside-out' signalling. The N-terminal regions of the α and β subunits form a globular headpiece region containing the binding site for integrin ligands.[6,7] Studies of the αvβ3[6–8] and αLβ2[9] integrins show that large-scale rearrangments in the structures of the external and cytoplasmic domains, respectively, of the integrins occur upon activation. Thus, in the resting state, the extracellular domains of αvβ3 resemble a 'closed switchblade', where some 4000-Å^2 of solvent-accessible surface, including ligand-binding regions, are occluded.[6,8] Activation extends the integrin structure and makes the binding site available as a 'closed' headpiece. Binding of ligand 'opens' the headpiece region.[7] The cytoplasmic domains of αL and β2[9] (or αIib[10] and β3[8]) chains are, in the resting state, very close together and intertwined in a 'handshake'-type structure. Ligand binding, signalling to the integrin via other pathways, or binding of the cytoplasmic protein talin, all cause significant spatial separation of the cytoplasmic domains (i.e. disruption of the handshake).

CHEMOKINES REGULATE B-LYMPHOCYTE POSITION AND INTEGRIN AFFINITY

Chemokines regulate recruitment and retention at many stages of B lymphopoiesis. In the bone marrow, the stromal cell-derived chemokine, stromal-cell-derived factor-1 (SDF-1)/CXCL12, acting via the CXCR4 receptor,[11,12] attracts CD34$^+$ progenitor cells to the stromal microenvironment.[13] CXCL12 also enhances the affinity of lymphocyte function-associated antigen-1 (LFA-1), very-late activation antigen (VLA)-4 and VLA-5 integrins expressed by CD34$^+$ progenitors,[14] thus ensuring that the cells are retained on the stromal surface. As developing B cells progress to the mIgM$^+$ immature B-cell stage, sensitivity to CXCL12,[15] but not CXCR4 expression,[16] is lost and the cells escape the bone marrow and enter the periphery. Mice

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lacking either CXCL12 or CXCR4 have impaired B lymphopoiesis and myelopoiesis, and neurological development is also perturbed.[17–19]

Chemokines and integrins also have critical roles in B-cell movement between and within secondary lymphoid tissue. The spleen is divided into red and white (lymphoid) pulp, the latter having discrete inner T-cell zones and outer B-cell follicles. The marginal zone (MZ) – the boundary between white and red pulp – surrounds the follicles and is rich in macrophages, reticular cells and B cells. Lymphocytes require both LFA-1 (αLβ2) and α4β1 integrins for entry into the spleen[20] via interactions with intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), respectively, and recirculate continuously through the white pulp. CCL19 and CCL21, acting via CCR7, mediate recruitment of T cells and dendritic cells to T-cell areas,[21,22] while CXCL13 and its complementary receptor, CXCR5, are mandatory not only for B-cell migration into splenic B-cell follicles, but also for development of lymph nodes and Peyer's patches.[23] In an immune response, antigen-activated B cells move quickly to the region corresponding to the interface between the T and B zones.[24] After B-cell receptor (BCR) signalling, B cells express CCR7, but do not reduce CXCR5 levels, and migrate to the T/B-zone boundary under the influence of CCL19 and CCL21. The B cells respond less strongly than T cells, a feature that may explain their accumulation at the edge of the T-cell zone rather than infiltration of the area. In contrast to the B-cell follicles, the MZ B-cell population is largely non-recirculating. Entry of B cells into the MZ is dependent upon BCR signalling, but is independent of CXCL13.[25] MZ B cells have higher LFA-1 and α4β1 levels than follicular B cells, and this may explain why MZ B cells display extended non-recirculating behaviour.[25] Indeed, release of B cells from the MZ requires loss of integrin function.

THE α6-INTEGRINS AND THEIR POSSIBLE ROLES IN B-CELL FUNCTION

The α6 chain is found in only two heterodimeric combinations, α6β1 and α6β4. The α6β1 integrin (also known as VLA-6) binds laminins-1, -2 and -4, while α6β4 binds laminin-1 and, with higher affinity, laminin-5. The α6 subunit has two splice variants, α6A and α6B,[4] each with a unique cytoplasmic domain but no difference in laminin specificity. The α6β4 integrin is found mainly in hemidesmosomes, and the large cytoplasmic domain of the β4 integrin[26] is important in the integrity of these structures. Mice lacking either α6[27] or β4[28] genes display perinatal lethality and have pronounced skin blisters ($\beta1^{-/-}$ mice die shortly after implantation).[29] In humans, mutations in α6 or β4 are associated with epidermolysis bullosa.[30]

There are very few studies of the α6-integrin in the context of specific immune responses, and the report by Ambrose & Wagner,[1] in the current issue of *Immunology*, shows convincingly that GC B cells express high levels of the α6-integrin in a functional, laminin-specific receptor. GC B cells [peanut agglutinin-positive (PNA^+)] have a sixfold higher level of α6-integrin than naïve, non-GC B cells (PNA^-) in both Peyer's patch and spleen. Signalling via the BCR or CD40 promotes α6 expression by splenic B cells, although interleukin-4 (IL-4) is without effect. The A20 B-cell line, representative of GC B cells, expresses high levels of the α6A isoform of the integrin, and the α6-integrins expressed by both A20 and GC B cells are assembled into specific and functional laminin receptors. Intriguingly, anti-α6 immunoglobulin not only prevents adherence of A20 cells to laminin-coated plates, but also doubles the number of cells in S-phase, suggesting a link to cell growth and survival.

The authors favour a model where α6-integrins gain the high-affinity configuration in the light zone and so retain centrocytes in an environment where survival signals from follicular dendritic cells and T helper (Th) cells are available. What signals might regulate α6-integrin affinity in the GC? That BCR or CD40 signalling can promote α6 expression is not disputed, but a different signal, originating in the GC itself, may be a more likely mechanism to regulate α6-intergin activation. The CXCL13/CXCR5 interaction is a strong candidate,[23] but as BCR and CD40 signalling promote CXCR7 expression by naïve B cells,[24] CCL19 and CCL21 derived from Th cells in the GC light zone must also be considered. Moreover, signalling via the BCR and CD40 is mandatory for centrocyte survival[31] and represents success in terms of selection. Therefore, in centrocytes, BCR and CD40 signalling might promote either loss of α6 expression or reversion to the low-affinity state. Selected centrocytes lacking α6 function would no longer be tethered to the laminin network and could migrate out of the GC light zone. This is similar to the requirement for loss of α4β1 function in MZ B cells to allow their egress from that site[25] and it is also interesting that the GC cells studied in this report appear to have reduced α4 integrin levels, a feature that would not favour retention in the MZ.

The precise α6 heterodimer(s) expressed by GC B cells remains to be defined unequivocally and, because α6β1 and α6β4 have different laminin specificities, this may be important in the context of laminin isoforms available in the GC light zone. It is unclear at this stage whether α6β1, α6β4 or both integrins mediate the retention function proposed. Although the flow cytometry data suggest that β4 levels are twofold higher in GC B cells than in naïve B cells, it is not clear if this is reflected in the surface expression of α6β4. The cytoplasmic domain of the β4 subunit, by virtue of its large size,[26] offers greater potential for recruitment of cell-type or differentiation-state-specific signalling effectors than the shorter β1 domain. Moreover, the interaction between α6 and β4 is monogamous, whereas β1-integrin is found in 11 heterodimers (see reference 3), so tightly regulated expression of α6β4 may offer a greater chance of differentiation stage-specific responses than the more ubiquitous α6β1. The α6 isoform expressed is also important, and the data from the A20 line suggest that the α6A isoform is expressed preferentially in GC B cells. While this does not influence laminin binding, α6A and α6B have different cytoplasmic domains that could influence GC B-cell responses.[4] Although ablation of α6 causes perinatal lethality,[27] a mouse line that expresses α6B, but not α6A, is viable and largely phenotypically normal apart from a selective defect in lymphocyte distribution.[32] The $\alpha6A^{-/-}$ mice show normal homing of lymphocytes to secondary lymphoid tissues *in vivo*, but isolated T cells display a notable reduction in migration in response to CXCL12 *in vitro*.[32] No analysis of any B-cell function in the

$\alpha 6A^{-/-}$ animals was presented, but the studies from the Wagner laboratory[1] clearly provide a new impetus to evaluate GC formation and centrocyte survival in detail in the $\alpha 6^{-/-}$ mice.

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