The value of gene ‘knock-out’ for assessing the role of cell adhesion molecules in renal disease

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Background: why ‘knock out’?

A number of approaches have been used to determine the relative contributions of the four main classes of leukocyte adhesion molecules (selectins/selectin counter-receptors, integrins/immunoglobulin superfamily members) to the pathophysiology of autoimmune renal inflammatory disease, allograft rejection, and ischaemic acute renal failure (ARF). These strategies include administration of monoclonal antibodies, soluble forms of adhesion molecules, synthetic oligosaccharides, and antisense oligonucleotides [1,2]. Most investigators have employed monoclonal antibodies against key adhesion epitopes. While this strategy has provided useful information in vivo and in vitro, certain methodological shortcomings mandate that any results must be interpreted cautiously. The administration of monoclonal antibodies per se could potentially perturb the physiological/pathophysiological scenario and give a false impression of protection by (i) promoting lysis of critical leukocyte subsets, (ii) activating and subsequently desensitizing leukocytes by mobilizing signal transduction pathways through specific antigen–antibody interactions (so-called ‘outside-in’ signalling), and (iii) perturbing of cellular function via non-specific ligand interactions. Moreover, administered monoclonal antibodies maybe insufficient to inhibit the function of specific adhesion molecules either due to dosing limitations or as a result of the inaccessibility of the exposed molecule. An alternative approach which has gained increasing favour over the past 5 years is the deletion of functional adhesion molecules through homologous recombination in embryonic stem cells: gene ‘knock-out’. This technology has the advantage of deleting all active molecules. Potential disadvantages of gene-targeting include its current restriction to mice and the potential which exists for breeding-based selection of ‘knock-out’ animals that have survived gestation through compensatory amplification of other adhesion molecules/classes. Studies have indicated that chronic deficiency of some adhesion molecules in ‘knock-out’ mice results in the perturbation of basal and cytokine-induced endothelial cell surface expression of other adhesion-mediating entities [3]. Notwithstanding this caveat, investigations employing adhesion molecule-deficient animals have significantly advanced our understanding of the function of these leukocyte adhesion molecules in renal disease. Before discussing these studies, it is useful to set the stage by briefly reviewing the paradigm of leukocyte trafficking/immune cell infiltration as determined using monoclonal antibodies.

Paradigm of leukocyte trafficking as determined using monoclonal antibodies

The movement of immune cells from the blood vessel lumen to an inflammatory site occurs via an orchestrated process involving chemotaxis, adhesion to endothelium (margination), penetration of endothelial cell tight junctions and migration through endothelium (diapedesis), basement membrane, and extravascular tissue [1]. Complementary in vitro and in vivo studies using monoclonal antibodies suggest that initial leukocyte attachment (rolling) is mediated by engagement of selectins and their carbohydrate counter-receptors, whereas endothelial adhesion and transendothelial migration are facilitated by interactions between leukocyte integrins and endothelial cell immunoglobulin-like (Ig-like) counter-receptors [1,2]. ‘Knock-out’ mice have enabled this paradigm to be tested through an alternative technology.

Selectin-deficient mice

Role of P-selectin in glomerulonephritis (GN)

In support of the importance of P-selectin in renal pathophysiology, glomerular endothelial expression of this molecule has been reported within 30 min of induction of complement-independent murine nephrotoxic serum nephritis [4]. These workers also noted a close temporal relationship between the peaks of P-selectin expression and neutrophil (PMN) recruit-
ment, and demonstrated that the prior administration of a blocking anti-P-selectin antibody ablated neutrophil recruitment by over 90%. Monoclonal antibody did not, however, influence either neutrophil influx or proteinuria in two alternative rat models of acute immunocomplex GN [5,6]. Against this backdrop, Mayadas et al. studied nephrotoxic serum nephritis in P-selectin deficient mice [7]. Homozygous P-selectin ‘knock-out’ mice have elevated numbers of circulating neutrophils under basal conditions, total absence of leukocyte rolling in mesenteric venules and delayed recruitment of neutrophils to the peritoneal cavity following thioglycollate administration [8]. Delayed hypersensitivity is also attenuated in these animals. Surprisingly, induction of active passive anti-glomerular basement membrane (GBM) nephritis in P-selectin deficient mice led to an increase in both PMN recruitment and proteinuria relative to wild-type animals [7]. To reconcile these intriguing and apparently contradictory results it is important to consider the differing roles of endothelial and platelet P-selectin. In addition to mediating neutrophil adherence to the endothelium, P-selectin also regulates PMN-platelet interactions. Indeed, P-selectin is present at 13-fold greater concentrations on platelets as compared to endothelial cells [9]. PMN-platelet adhesion through P-selectin is a major feature of rat con A-F GN [6], murine nephrotoxic serum nephritis [7] but not of murine anti-GBM nephritis in which monoclonal anti-P selectin administration proved protective [4]. During P-selectin dependent adhesion of PMN and platelets, these cells effectively pool their enzymatic machinery to generate lipoxins a novel class of eicosanoids that inhibit PMN recruitment in several in vitro and in vivo models. In P-selectin ‘knock-out’ mice transcellular biosynthesis of lipoxins is significantly reduced [7] leading these authors to speculate that the ‘braking’ action of these eicosanoids on PMN recruitment is reduced in P-selectin deficient mice, hence the enhanced neutrophil infiltration [7,10]. In conclusion, studies of GN in P-selectin deficient mice underscore the importance of considering the consequences of P-selectin mediated PMN-platelet interactions as well as associations between PMN and endothelial cells in inflammatory disease, and highlight the complex role of this adhesion molecule in renal inflammation.

Role of L-selectin in ARF

L-selectin-deficient mice exhibit impaired leukocyte rolling and reduced migration into the peritoneum in classic models of inflammation [11]. The influence of L-selectin blockade on ARF has been the subject of one study to date. Intertubular PMN infiltration and ARF were indistinguishable in ‘knock-out’ and wild-type animals [12]. These results suggest that L-selectin is not crucial to PMN recruitment in ARF. The influence of L-selectin ‘knock-out’ on GN has not been studied, this is warranted, however, given in vitro evidence that cytokine-activated glomerular endothelial cells express ligand(s) for L-selectin [13].

E-selectin deficient mice

While deletion of L-selectin alone disrupts leukocyte trafficking mechanisms, studies in E-selectin ‘knock-out’ mice have been informative in highlighting overlap of function between endothelial P-selectin and E-selectin with respect to regulation of initial loose tethering and rolling of leukocytes [14]. The influence of this phenotype on the course of GN and ARF has not been reported yet.

Role of CD11b/CD18-2, ICAM-1-, and VCAM-1-deficient mice

Role of CD11b/CD18 and ICAM-1 in GN

In addition to its cognate ligands, CD11b/CD18 has been shown in vitro to functionally interact with Fcγ receptors and thereby facilitate immune complex (IC)-stimulated PMN functions [15]. The induction of acute Fc-dependent anti-GBM nephritis in CD11b/CD18-deficient mice resulted in equivalent initial glomerular PMN accumulation between these and their wild-type counterparts. However, at later timepoints (2 h post-injection) neutrophil accumulation decreased in mutant mice and increased in control animals [16]. Parallel in vitro studies showed that spreading of CD11b/CD18-deficient PMN to IC-coated dishes also exhibited a time-dependent reduction, leading the authors to speculate that in vivo CD11b/CD18 is not necessary for Fc-mediated PMN recruitment but that CD11b/CD18-FcγR associations are an essential prerequisite for the filamentous actin reorganization which results in sustained PMN adhesion. Interestingly, in attempting to determine the mechanism underlying the observations in nephritic CD11b/CD18-deficient mice, these authors also generated the disease in ICAM-1 and C3 gene targeted animals. Results obtained in studies using monoclonal antibodies have reported protective anti-ICAM-1 effects in various rodent models of GN [1]. By contrast, no significant differences in glomerular PMN counts were observed between ICAM-1 and C3 ‘knock-outs’ and wild-type mice, suggesting that in this scenario at least, CD11b/CD18-ICAM interactions are less relevant than CD11b/CD18-FcγR associations. The observation that both CD11b/CD18- and C3-deficient animals exhibited significantly reduced proteinuria than control mice, prompted the authors to propose a model wherein CD11b/CD18–ic3b interactions are required for neutrophil release of GBM-damaging azurophilic granules.

Role of ICAM-1 in ARF

Whereas as already noted, ICAM-1 deficiency did not influence the course of acute GN in the single report to date, evidence arising from studies on ICAM ‘knock-out’ animals have indicated that this Ig-like adhesion molecule contributes to PMN infiltration in ARF. ICAM-1 deficient animals were protected from ARF induced by occlusion of the renal pedicle, a benefit
that correlated with markedly reduced renal leukocyte infiltration [17]. In order to further evaluate whether this protective effect resulted from ineffective PMN infiltration, neutrophil-depleted mice were generated and, indeed, these animals were also ARF-resistant. These results are compatible with previous studies using function blocking ICAM-1 antibodies in ARF in rodents [18]. It should be noted, however, that anti-ICAM-1 monoclonal antibodies do not protect against ischemia–reperfusion injury in rabbits, suggesting that there may be marked differences in the pathophysiology of ARF between species [18].

**VCAM-1-deficient mice**

VCAM-1 ‘knock-out’ is a lethal phenotype due to impaired placentation and cardiac maldevelopment [19]. These observations highlight the capacity of ‘leukocyte’ adhesion molecules to perform functions independent of immune cell trafficking. Indeed, given that VCAM-1 is constitutively expressed in abundance by glomerular parietal epithelial cells [1], it is likely that this molecule also plays important non-immune roles in the kidney.

**Conclusion**

In conclusion the advent of gene-targeting technology has advanced our understanding of the role of adhesion molecules in renal disease. In some cases ‘knock-out’ mice confirmed previous studies using monoclonal antibodies, while in others this technology suggested novel roles for leukocyte adhesion molecules in GN and ARF. The ‘knock-out’ strategy is a powerful addition to the repertoire of tools currently being used to tease out the relative contributions of leukocyte adhesion molecules to the pathology of renal disease.

**References**