The Pathophysiologic Role of $\alpha 4$ Integrins In Vivo

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Introduction

The integrins $\alpha 4\beta 1$ (very late antigen-4: VLA4; CD49d/CD29)[1] and $\alpha 4\beta 7$ are cell surface heterodimers expressed mostly on leukocytes. The VLA4 molecule, initially characterized on lymphoid cells, was subsequently shown to mediate cell adhesion to vascular cell adhesion molecule-1 (VCAM1) (CD106), as well as to an alternately spliced form of the extracellular matrix protein fibronectin (Fn) (for review see references 1–4). VCAM1 was originally described as an inducible endothelial cell adhesion molecule, but has subsequently been found to be constitutively or inducibly expressed on many other cell types (2, 3). The integrin $\alpha 4$ can associate with another β subunit, first called βP in the mouse (5) and now designated $\beta 7$. Integrin $\alpha 4\beta 7$ appears central to lymphocyte homing to intestinal tissue via adherence to the gut homing receptor mucosa addressin cell adhesion molecule (MadCAM) (6) and binds also to VCAM1 and Fn (7, 8). These functional activities defined in vitro suggested that $\alpha 4$ integrins might play critical roles in migration of leukocytes into tissues at sites of inflammation.

In the past few years specific monoclonal antibodies which block $\alpha 4$-dependent adhesive function in vitro have been tested in vivo. In 1991 and 1992, only a few papers were published using such mAbs, but in 1993 there were 15, with many more either published or in press so far this year (Table I). Here we review these rapidly accumulating in vivo data which suggest that $\alpha 4$ integrin–dependent adhesion pathways are critical intervention points in several inflammatory and autoimmune pathologies. To save space we have been unable to cite all original references, but these can be found within either recent reviews (2–4) or the more recent references given.

Overview of $\alpha 4$ integrin distribution and in vitro functions

The VLA4 integrin is expressed at substantial levels on most mononuclear leukocytes, whether in circulation, within lymphoid organs, or resident in other tissues (1). Also it is found on eosinophils, basophils (9), and various nonhematopoietic tumor cells (e.g., rhabdomyosarcoma, melanoma). The $\alpha 4\beta 7$ integrin is expressed on most lymph node T and B cells (10), on the gut homing subset of $CD4^+$ memory T cells (11), and on lymphocytes resident in rheumatoid synovium (12). Recent studies show that natural killer cells, eosinophils, and newborn blood B and T cells show relatively homogeneous expression of $\alpha 4\beta 7$, while adult blood B cells and $CD8^+$ T cells, like $CD4^+$ T cells, show more heterogeneous expression (13). Finally, the $\alpha 4$ subunit (with unspecified β) is found in several nonlymphoid tissues in the developing embryo, including vascular smooth muscle and skeletal muscle (14, 15).

VLA4 recognizes a motif containing the sequence EILDVPST within the alternately spliced connecting segment 1 (CS1) region of Fn (3), with the LDV sequence being the most critical (16). VLA4 binds to sites within the first and fourth immunoglobulin-like domains of the full-length seven-domain form of VCAM1 (17, 18). Within domain one, a QIDSPL motif appears to be critical to integrin recognition (19, 20). Within the VLA4 molecule, binding sites for the CS1 region of Fn and for VCAM1 are overlapping, as evidenced by antibody cross-blocking (21) and competitive binding studies (22). However, these binding sites have distinct features, since the VLA4 interaction with VCAM1 but not Fn is supported by calcium ions (23) and some antibodies selectively inhibit only the latter adhesive interaction (24).

A number of weaker VLA4 interactions have been reported, for example with the Fn HepII (25), CS5 (26), and RGD (27) sequences, as well as with thrombospondin (28), but these interactions generally require VLA4 to be highly activated and their in vivo relevance remains to be determined. VLA4 (among other integrins) also interacts with the bacterial coat protein invasin (23, 29).

The $\alpha 4\beta 7$ ligands Fn, VCAM1, and MadCAM apparently bind to overlapping sites within the $\alpha 4\beta 7$ molecule, but these three interactions respond quite distinctly to regulatory antibodies and divalent cations (6, 11). The complete absence of any VLA4 reactivity with MadCAM (6) implies a distinct role for the $\beta 7$ chain not shared by the $\beta 1$ chain.

Like other integrins, both VLA4 and $\alpha 4\beta 7$ can exist in a range of activation states, depending both on cell type and on the extent of triggering by various cellular agonists such as phorbol esters, anti-CD3 antibodies, chemokines, and chemotaxis factors (6, 23, 30–32). Whereas VCAM1 has a low threshold of activation (23) and can support constitutive adhesion by PBLs (33), adhesion to Fn requires a higher level of activation (23, 34), thus reducing constitutive interaction between blood cells and soluble Fn in plasma. Cells bearing $\alpha 4\beta 7$ adhere avidly to MadCAM (6).

The cytoplasmic domain of $\alpha 4$ plays a key role in regulating cell adhesion (35), with the five to six residues just after the

Biogen, Inc. has a commercial interest in the development of VLA4-based therapeutic.

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1. *Abbreviations used in this paper:* AHR, airways hyperresponsiveness; BAL, bronchoalveolar lavage; CS, connecting segment; DTH, delayed-type hypersensitivity; EAE, experimental allergic encephalomyelitis; Fn, fibronectin; MadCAM, mucosal addressin cell adhesion molecule; NOD, nonobese diabetic; VCAM1, vascular cell adhesion molecule-1; VLA, very late antigen.

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Table I. In Vivo Studies with mAbs to α4 Integrins

Type of study	Species	mAb	Reference
Cell recruitment			
Lymphocyte	Mouse, rat	R1-2, PS/2, TA-2, HP2/1	58, 59, 66–68, 71, 74–77
Eosinophil	Guinea pig	HP1/2	60, 71, 82, 83
Monocyte	Rabbit	HP1/2	61, 95
PMN	Rat, rabbit	TA-2, HP1/2	61, 69
Disease model			
Lung antigen challenge	Mouse, rat, guinea pig, sheep	PS/2, TA-2, HP1/2	62, 71, 81–83
Ulcerative colitis	Primate	HP1/2	63
EAE	Mouse, rat	R1-2, HP2/1	59, 67
Contact hypersensitivity	Mouse	R1-2, PS/2	72, 73
Diabetes	Mouse	R1-2, PS/2	74–76
Nephritis	Rat	HP2/1, TA-2	79, 80
Allograft rejection	Rat, rabbit	TA-2, HP1/2	70, 95
Other studies			
Progenitor mobilization	Primate	HP1/2	64
Intestinal infection	Rat	TA-2	91
Gut homing	Mouse, rat	R1-2, PS/2, DAK32, TA-2	58, 77

transmembrane region being most critical (36). Compared with VLA4, the α4β7 integrin may have specialized regulatory features, including a greater requirement for phorbol ester stimulation (7, 8), and distinctive regulation through the β7 cytoplasmic domain (37).

Adhesion through VLA4 can lead to a wide spectrum of subsequent events. Both Fn and VCAM1 can act through VLA4 to deliver costimulatory signals (together with anti-CD3/T cell receptor) leading to T cell proliferation and cytokine production (38, 39). Likewise, adhesion through VLA4 may promote (40) or inhibit (41) cell death, depending on the lymphoid cell type and other conditions; triggering through VLA4 can regulate expression of genes for T cell 72-kD gelatinase (42), and monocyte inflammatory mediators (43), and may trigger tyrosine phosphorylation of a 105-kD protein in lymphocytes (44, 45). A further consequence of VLA4/ligand interaction is the transendothelial migration of monocytes (46, 47), the random migration of lymphocytes through filters coated with VCAM1 or Fn (48), and the migration of lymphoma cells beneath stromal cells (49). Consistent with these results, the α4 cytoplasmic domain may be particularly well suited to support cell migration (50).

α4 integrin structure and mAb epitopes

The α4 gene encodes a 150-kD protein and has been cloned from both murine and human sources (51, 52). Its primary sequence shows most similarity (39%) to the integrin α9 subunit (53). The mature 150-kD α4 protein can be variably cleaved into 80- and 70-kD fragments, but this cleavage does not alter adhesive functions (54). Another unusual feature of α4 is a conformational rearrangement, dependent on divalent cations and certain critical cysteines, that causes it to migrate at 180 kD instead of 150 kD in SDS gels (reference 55 and Pujades, C., and M. E. Hemler, manuscript in preparation).

The majority of monoclonal antibodies recognizing the human α4 subunit define three nonoverlapping epitopes (21). mAbs to epitope A partially block VLA4 adhesion to Fn but do not inhibit VCAM1 adhesion, whereas mAbs to epitope B effectively block adhesion to both ligands (as well as to invasin). Also, mAbs to epitope A and a subset of those to epitope B trigger homotypic aggregation (21). Epitope C mAbs have no effect on either adhesion or aggregation. Antibodies that recognize the human VLA4 (56) and α4β7 (11) complexes have also been described and should prove useful in distinguishing the two heterodimeric forms. Both function-blocking and nonblocking anti–mouse α4 antibodies have also been defined, as well as an antibody, DAK32, that specifically recognizes the murine α4β7 complex (6).

Importantly, mAbs that block adhesive function in vitro have now been characterized which work in all species, allowing in vivo studies to be performed in a variety of animal models (Table I). These include rat anti-murine α4 mAbs R1-2 and PS/2 (5, 57), both of which can induce homotypic aggregation (Holzmann, B., personal communication), murine anti–rat α4 mAb TA-2 (58), murine anti–human mAb HP2/1 (21), which binds and blocks rat α4 (59), and murine anti–human mAb HP1/2 (21), which binds and blocks guinea pig, rabbit, sheep, and primate α4 (60–64).

In vivo studies with mAbs to α4

mAbs to α4 block leukocyte recruitment. The identification of VLA4 as a counterreceptor for VCAM1 (65) suggested that this adhesion pathway might play a role in migration of VLA4-expressing cells from blood to tissues at sites of inflammation, and several studies have confirmed that this is indeed the case (58–61, 66–68). The earliest studies were those of Issekutz and co-workers (58, 66), who showed that the migration of ^{111}Indium-labeled rat lymphocyte subpopulations into inflammatory sites in the skin, both in response to cytokines and to a classic delayed-type hypersensitivity (DTH) reaction, and in the joints was α4 integrin dependent. In another study, eosinophil recruitment was evaluated (60) and mAb HP1/2 was found to block 50–80% of cellular recruitment into guinea pig skin in response to a variety of mediators and to a passive cutaneous anaphylaxis reaction.

The lack of expression of VLA4 on PMN suggests that

blockade of this integrin should not block acute PMN emigration and this is the case in several in vivo systems. For example, mAb HP1/2 does not block PMN recruitment at 4 h into rabbit peritoneum in response to protease peptone (61) and mAb TA-2 does not affect acute PMN-dependent complement-mediated lung injury in the rat (69). However, mAbs to $\alpha 4$ can affect PMN recruitment indirectly. For example, PMN-dependent edema is reduced in the mouse ear in T cell–dependent contact hypersensitivity (see below), and mononuclear leukocyte–dependent PMN recruitment into rabbit peritoneum is inhibited at 24 h after protease peptone administration (61).

Several studies have looked at the combination of $\alpha 4$ mAbs with either CD18-directed or lymphocyte function-associated antigen-1–directed mAbs (61, 68, 70, 71). Importantly, mononuclear leukocyte recruitment is almost completely abolished in the majority of models examined, independent of species, mAb used, or organ examined.

The data in many of these studies were interpreted to mean that VLA4 plays a critical role in leukocyte recruitment in vivo. However, with the increasing recognition that many leukocytes also express integrin $\alpha 4\beta 7$ (13), the relative role of each $\alpha 4$ integrin (as well as the nature of the counterligands involved) remains undefined in many systems and must be assessed in further studies (see following section). Nevertheless, the data argue that $\alpha 4$ integrins play a central role in leukocyte emigration from peripheral blood into inflammatory sites and that $\alpha 4$ and $\beta 2$ (CD18) integrins combine to coordinate leukocyte emigration into most tissues and organs in the body.

mAbs to α4 in rodent models of disease. mAbs to $\alpha 4$ have shown therapeutic effects in numerous rodent models of disease, including three classic models of T cell–dependent autoimmune disease. The first reports used rat and mouse models of experimental allergic encephalomyelitis (EAE) (59, 67), induced by passive transfer of $CD4^+$ myelin basic protein–specific T cell clones. After migration of these cells within 4–12 h into neural tissue and generation of an inflammatory response, hind limb and tail paralysis occurs at 4–5 d after injection. Yednock et al. (59) first showed that a single intraperitoneal injection of mAb HP2/1 on day 2 after passive transfer significantly delayed onset of paralysis in rats. The mAb had no effect on blood cell counts, and brainstems from control animals revealed extensive leukocyte infiltration, while such infiltration was absent from treated animals. Because the mAb was administered at day 2, i.e., after the entry of the T cell clones into neural tissue, it was proposed that mAb treatment blocked entry of host mononuclear leukocytes recruited nonspecifically to the site of inflammation. A second study in a mouse EAE model (67) confirmed and extended these results. These authors showed that the $CD4^+$ T cell clone could be further subdivided by phenotype. First, TH1 but not TH2 clones could induce disease. Second, induction of disease correlated with surface expression of $\alpha 4$ integrin. They found that coinjection of cells with either mAb R1-2 to $\alpha 4$ or mAb MK/1 to murine VCAM1 significantly delayed onset of paralysis. The data from both studies are consistent with VCAM1 on inflamed brain endothelium recruiting both antigen-specific T cells and nonspecific leukocytes into neural tissue via VLA4 (see below).

T cell–dependent murine contact hypersensitivity models (72, 73) also demonstrate a role for $\alpha 4$ integrins. Intravenous administration of either mAb R1-2 or PS/2 4–6 h before challenge inhibited 50–60% the ear swelling response of mice sensitized with either dinitrofluorobenzene or oxazalone (73). In a second study, mAb R1-2 blocked by ~ 80% the ear swelling induced by the adoptive transfer of trichloronitrobenzene-sensitized spleen cells (72). Interestingly, mAb R1-2 did not inhibit the overall emigration of either nonimmune or immune T cells (72), suggesting that in this model the mAbs do not function by inhibiting recruitment.

A third model of T cell–dependent autoimmune disease in which $\alpha 4$ mAbs have been evaluated is the nonobese diabetic or NOD mouse, which spontaneously develops type I diabetes, characterized by infiltration of pancreatic islets (insulitis) and destruction of insulin-producing islet cells. Three independent studies have shown that mAbs R1-2 (74–76) or PS/2 (75) both inhibit insulitis and delay significantly the onset of diabetes. mAbs MK/1 and MK/2 identify VCAM1 on inflamed but not normal islet vessels and also block onset of disease when used in vivo (75, 76).

A role for $\alpha 4\beta 7$ cannot formally be ruled out in these three models. However, recent studies show that, while mAb PS/2 blocks $\alpha 4\beta 7$-dependent lymphocyte adhesion in vitro and blocks lymphocyte homing to the gut, mAb R1-2 does neither (6, 77). In contrast, mAb R1-2 does block VLA4-dependent adhesion in vitro (8, 77). The efficacy of R1-2 in all three models, combined with the pan-lymphocyte distribution of VLA4 and the more restricted distribution of lymphocyte $\alpha 4\beta 7$, strongly argues that R1-2 is in fact a VLA4-specific mAb in vivo and that VLA4 is indeed the leukocyte receptor. The ability of mAbs to VCAM1 to block in the models in which they were tested (67, 75, 76) also points to VCAM1 as the VLA4 counterreceptor, at least on brain and islet endothelium.

In addition to these murine studies, mAb TA-2 has also been used in several rat models and has implicated $\alpha 4$ integrins in vascularized cardiac allograft rejection (70), immune complex–mediated lung injury (78), acute nephrotoxic nephritis (79), and in skin induration and fibrin deposition in DTH reactions (68). In addition, mAb HP2/1 significantly inhibits mercuric chloride–induced nephritis in Brown Norway rats (80).

mAbs to α4 in allergic lung inflammation. Several in vivo studies have now been performed in different species examining the role of $\alpha 4$ integrins in allergic airways (62, 71, 81–83). In a sheep model of allergic asthma (62), animals challenged with the parasite *Ascaris suum* undergo acute bronchoconstriction. Importantly, many animals then show a late phase response (LPR) 6–8 h after challenge, which correlates with eosinophil-rich leukocyte infiltration into the lung. mAb HP1/2 was highly effective at blocking the LPR, as well as the associated airways hyperresponsiveness (AHR) to carbachol (62). Nevertheless, inhibition of cellular recruitment could not fully explain the data, because bronchoalveolar lavage (BAL) leukocyte levels were affected to only a small degree by mAb treatment. Interestingly, aerosolized mAb HP1/2 was as effective as intravenous HP1/2 in blocking both the LPR and AHR, suggesting that the therapeutic effects in this model are due to mechanisms operative within the lung itself.

Consistent with the sheep data, treatment of ovalbumin-sensitized Brown Norway rats with mAb TA-2 just before challenge significantly blocked the LPR without significant changes in BAL leukocyte composition (81). In contrast, blockade of $\alpha 4$ integrin with mAb PS/2, or VCAM1 with mAb MK/1, significantly inhibited both eosinophil and T cell recruitment into the tracheas of ovalbumin-sensitized and challenged mice (71), which strongly express VCAM1 as assessed by immunohistology.

In ovalbumin-sensitized guinea pigs, Pretolani et al. (82) have shown that mAb HP1/2 effectively blocks AHR in response to carbachol after challenge. In this study, reduced eosinophil numbers are seen in BAL fluid. Immunohistologic studies of lung tissue show significantly reduced levels both of eosinophils and of both $CD4^+$ and $CD8^+$ T cells in the epithelial submucosa and adventitia (82). However, another study (83) found that, despite reduced eosinophil numbers and eosinophil basic peroxidase levels in the BAL of HP1/2-treated animals, the mAb had no effect on AHR to acetylcholine. The reasons for this discrepancy are unclear at present.

Taken jointly, the data argue that $\alpha 4$ integrins likely play multiple complex roles in lung pathobiology, including both recruitment and adhesion-dependent priming or activation of leukocytes and that $\alpha 4$ integrin-dependent adhesion pathways may prove to be suitable intervention points for allergic asthma.

mAbs to $\alpha 4$ in inflammatory bowel disease. The cotton-top tamarin is a New World primate that experiences a spontaneous chronic colitis marked by periodic flares of acute inflammation that closely mimics human ulcerative colitis, one of the two major forms of inflammatory bowel disease (63). Animals were treated during acute flares with mAbs to either E-selectin or $\alpha 4$ integrins. While no significant reduction in colitis activity was seen with two mAbs to E-selectin, the animals treated with mAb HP1/2 showed a highly significant attenuation of their colitis, as assessed histologically, and a statistically significant increase in weight.

mAbs to $\alpha 4$ peripheralize progenitor cells. Cellular interactions between hematopoietic cells and their stromal microenvironment in bone marrow are known to be central to their programmed maturation. Interactions between $\beta 1$ and $\beta 2$ integrins and their known ligands expressed on the earliest stem and progenitor cells have been implicated on the basis of in vitro studies (for review see reference 64). Recently, treatment of primates with blocking mAbs to CD18 or $\alpha 4$ have provided insight into the functional role of these adhesion pathways in vivo (64). Anti-$\alpha 4$ treatment, but not anti-CD18 treatment, resulted in a 100-fold selective mobilization of progenitors into the bloodstream. Peripheralization involved erythroid, myeloid, and mixed progenitors, was detectable 24 h after injection, and lasted beyond the final injection. In contrast, anti-$\beta 2$ treatment had no effect on the numbers of peripheral progenitors, despite increasing PMN counts significantly, demonstrating efficacy of the mAb. Progenitor numbers were also increased by an order of magnitude when mAb HP1/2 was given after 5 d of granulocyte colony-stimulating factor treatment (64). The data provide evidence for a role for $\alpha 4$ integrins in progenitor cell function and trafficking in vivo and may provide a novel clinical application for $\alpha 4$ integrin blockade, since the use of peripheralized stem and progenitor cells for autologous transplantation after chemotherapy and for gene therapy applications is becoming of increasing importance in clinical medicine (84).

Intervention in vivo with alternative antagonists

Although mAbs blocking the in vitro function of VCAM1 in mouse, rat, rabbit, and cynomolgus monkey have been described, their use in vivo to probe the role of VCAM1 has been limited. The use of mAbs to VCAM1 in murine EAE, diabetes, and lung recruitment was described earlier, and these mAbs have also been used in allograft rejection studies (85). A recent study also showed that F(ab')2 fragments of an mAb to VCAM1 significantly inhibited $CD2^+$ lymphocyte accumulation in response to tuberculin in a primate DTH response (86). The data so far suggest that VCAM1 indeed can play a role in leukocyte recruitment, as originally hypothesized (65, 87). Although absence of VCAM1 by immunohistology has often been used to infer lack of importance in pathology, it is in fact unclear whether VCAM1 is really absent from normal vessels or merely present at levels below detection by standard immunohistochemical methods. Interestingly, mAbs to VCAM1 were found to block monocyte transendothelial migration in vitro, despite undetectable levels of VCAM1 on human umbilical vein endothelial cells when examined by mAb staining (47). Since VCAM1 clearly can mediate signal transduction (see above), low levels may be sufficient to promote a migratory phenotype (42) in the absence of strong adhesion. The availability and wider in vivo exploitation of VCAM1-directed mAbs should help clarify these points.

Blocking mAbs to murine MadCAM and to both the murine $\beta 7$ chain and the $\alpha 4\beta 7$ complex are now available and should prove to be valuable reagents to dissect the role of alternative pathways in this species, as shown in recent elegant studies on gut lymphocyte homing (77).

Finally, Fn-derived peptides have shown efficacy in murine contact hypersensitivity and rat adjuvant arthritis models, as well as in tranforming growth factor-$\beta 1$ knockout mice (88–90). The mechanism of action of these peptides in these models remains undefined, although they are presumed to interact with and block VLA4, VLA5, or $\alpha 4\beta 7$.

Side effects of mAbs to $\alpha 4$

The in vivo studies published to date have been concerned with the demonstration of therapeutic efficacy, and, not surprisingly, little attention has been paid so far to possible side effects of $\alpha 4$ integrin blockade. Nevertheless, $\alpha 4$-dependent adhesion pathways must play physiologic roles in leukocyte biology, and, therefore, blockade of these pathways will presumably have deleterious effects on normal immune and inflammatory responses. Blockade of this integrin does not block acute PMN emigration (see above), and, therefore, $\alpha 4$ integrin blockade should not affect acute PMN-dependent clearance of infectious organisms. However, a recent publication shows that $\alpha 4$ mAbs can have deleterious effects on clearance of infectious organisms in the gut (91). Rats treated with mAb TA-2 are unable to effectively resolve intestinal nematode infections, which are cleared by T cell–dependent mechanisms, arguing that $\alpha 4$ integrins play a central role in several areas of lymphocyte-dependent intestinal immunity (91). Recent studies also show that intestinal invasion of mice with the bacterium *Yersinia enterocolitica,* which is also cleared by T cell–dependent mechanisms, is significantly enhanced in the presence of $\alpha 4$-directed mAbs (Autenreith, I., personal communication).

Other possible mechanism-based side effects, based on tissue distribution of VCAM1 and $\alpha 4$ integrins, might include effects on antibody formation, hematopoiesis, neural development, mucosal immunity, muscle development, and wound healing, and it is clear that the side effects of $\alpha 4$ integrin blockade require further study.

Mechanism of action of $\alpha 4$ mAbs in vivo

Although blockade of recruitment into tissues clearly occurs in vivo and provides an explanation for the disease-modifying effects of mAbs in certain models, it is also apparent that mAbs can block disease in the absence of recruitment blockade. The

most striking example of this is in the sheep model of allergic AHR, where mAb HP1/2 is effective as an aerosol (62). These observations raise important issues about the mechanism of action of these mAbs, which have all been selected on the basis of blockade of adhesive function in vitro. In fact, $\alpha 4$ integrin–dependent adhesion is likely crucial not only to recruitment but also to leukocyte function within tissues. Adhesion-dependent enhancement of leukocyte function is well established in vitro for the $\beta 2$ integrins, and recent studies show the same phenomenon for $\alpha 4$ integrins on both eosinophils and T cells (42, 92). Recent studies also show that eosinophil activation state rather than number is critical to increased AHR in vivo in the guinea pig (93). Therefore, inhibition of adhesion-dependent priming and/or activation of leukocytes, either during transendothelial migration or when within tissues, provides an explanation for mAb efficacy despite lack of inhibition of recruitment.

It is also clear that mAbs to $\alpha 4$ integrins can trigger or prime leukocytes in vitro, for example to release cytokines (43), which are known to modulate disease. For example, TNF will delay the onset of diabetes in NOD mice (75), and induction of monocyte TNF release by mAbs crosslinking $\alpha 4$ could provide an alternative rationale for efficacy observed in the NOD model (74–76). In addition, the effector functions of mAbs can play an important role in their in vivo efficacy profiles. Several approaches can be taken to address these issues. First, IgG fragments lacking effector function can be tested. Of most value are monovalent Fab fragments which cannot crosslink receptors. In fact, monovalent Fab fragments of HP1/2 show comparable efficacy with HP1/2 IgG in vivo in blocking sheep LPR and AHR (94). Second, isotype-matched nonblocking mAbs can be used as effective controls. For example, mAb B5G10, an $\alpha 4$ mAb which is the same isotype as mAb HP1/2 and which binds primate $\alpha 4$ but does not block adhesive function, does not peripheralize progenitor cells in baboon studies (Papayannopoulou, T., and R. R. Lobb, unpublished data). Third, mAb HP1/2 does not induce cytokine RNA expression in human monocytes in vitro (Haskill, S., L. Osborn, and R. R. Lobb, unpublished data). These results are all consistent with mAb HP1/2 working in vivo by blockade of $\alpha 4$ integrin–dependent adhesive function. In the mouse system, Fab fragments of PS/2 have been examined and still block $\alpha 4$-dependent function (77). Finally, the equal efficacy of VCAM1-directed and $\alpha 4$-directed mAbs in two murine models argues that blockade of adhesion is the mechanism of action in these cases. In conclusion, the data from several in vivo studies argue for a mechanism of blockade of adhesion-dependent function, but further examination of these issues will be of value.

Summary

In this review we have summarized the rapidly mounting evidence for a central role for the integrins VLA4 and $\alpha 4\beta 7$ in leukocyte pathophysiology. Five distinct $\alpha 4$ mAbs, each able to block $\alpha 4$-dependent adhesion in vitro, show beneficial effects in vivo in seven different species (mouse, rat, guinea pig, rabbit, sheep, and New-World and Old-World monkeys) and in a wide variety of organ systems, including colon, lung, skin, neural tissue, pancreas, peritoneum, and the vessel wall (Table I). A number of important issues remain to be addressed, including the relative importance of VLA4 and $\alpha 4\beta 7$ and of their counterligands VCAM1, Fn, and MadCAM, in most in vivo settings; alternative mechanisms for mAb efficacy other than adhesion blockade; poor understanding of side effects of $\alpha 4$ blockade; and the role of integrin signaling rather than adhesion in function. Nevertheless, the data argue that $\alpha 4$ integrins will likely play critical roles in both normal physiology and pathology in man. To examine this issue, a humanized IgG4 isotype of mAb HP1/2 has been generated which retains full in vitro potency and in vivo efficacy (Lobb, R., D. Leone, B. Pepinsky, P. Tempest, F. Carr, W. Abraham, and S. Nourshargh, unpublished data). This mAb will enter clinical trials in the near future to extend in vivo studies to humans and to identify clinical areas of value. This area of leukocyte adhesion biology promises to remain a fruitful area of research and should continue to provide critical clues as to intervention points in human disease.

Note added in proof. Since submission of this manuscript, an excellent review of endothelial-leukocyte adhesion has been published (96). In addition, we omitted a paper showing that treatment of mice with mAbs to either VLA-4 or VCAM-1 both prolonged cardiac allograft survival and greatly suppressed antibody titer to human gammaglobulin (97).

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