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BRIEF COMMUNICATION

Lymphocyte traffic is modified *in vivo* by anti-laminin antibody

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SUMMARY

Data emerging from recent *in vivo* and *in vitro* studies are pointing to basement membrane and other extra cellular matrix (ECM) components as likely determinants of specific lymphocyte entry and positioning in lymphoid tissues. In this report, the relevance of this notion is investigated in T-cell deficient (B) rat recipients of cardiac allografts, using an anti-laminin (LN) antibody as the probe. The 6-hr migration patterns of ^{111}In-labelled peripheral lymph node (PLN) cells were followed in groups of engrafted B hosts treated with rabbit anti-rat LN antibody or rabbit serum. The accumulation of adoptively transferred cells in PLN and cardiac allografts of recipients pretreated with anti-LN antibody was significantly decreased compared to controls ($P < 0{\cdot}05$ and $P < 0{\cdot}001$, respectively). The transferred cells also localized in slightly lower numbers in the spleens and lungs of anti-LN conditioned rats; no differences were seen in the clearance of lymphocytes from peripheral blood, or their sequestration in liver and kidney. These data provide the first *in vivo* evidence that an antibody against LN selectively affects lymphocyte traffic. The results reinforce the notion that basement membrane components could be critical in 'directing' lymphocyte migration *in vivo*.

Different approaches to the study of the regulation of lymphocyte traffic and positioning *in vivo*, have recently led to the consideration that basement membrane components could have an important role in this process.[1] Historically, the starting point of the experimental journey of the two dominant approaches to the study of lymphocyte circulation resides in the post-capillary venules (PCV) of the lymph nodes. One approach evolved round the conviction that the height of the endothelium was important for the selective entry of cells; the other developed from the early recognition that lymphocyte entry in lymph nodes occurs through PCV regardless of the height of the endothelial cell (reviewed by De Sousa, 1981).[2] Consequently, experiments designed within the first approach concentrated on interactions of lymphocytes with the so-called high endothelium venules (HEV); experiments designed along the second approach focused on interactions between lymphoid cells themselves and other structural components.[3] Recently, the utilization of cDNA clones encoding for endothelium binding molecules led to the discovery that one of them (CDw44/Hermes) is a member of the cartilage link protein family,[4,5] and that a second one (LPAM-1) has a structure virtually identical to that of the human VLA-4 integrin receptor.[6] The significant sequence homologies of CDw44 with cartilage link proteins indicate a role for collagen-mediated matrix adhesion.[1] The co-appearance of increased amounts of collagen and T lymphocytes in the synovial tissue of animals pretreated with ferric citrate led to the conclusion that collagen could be a unifying clue to the different mechanisms regulating specific entry and probably positioning of lymphocytes in well-defined micro-environments of the peripheral lymphoid organs (ecotaxis). This view is reinforced by studies of the adhesion of human peripheral blood T cells to collagen substrata *in vitro*, indicating that lymphocytes have collagen-binding surface components,[7] and that members of the VLA family of molecules mediate the binding of T cells *in vitro* to fibronectin (VLA-4 and VLA-5) and to laminin (VLA-6).[8]

In this study, the relevance of the newly found clue was tested in T-cell deficient (B) rats, using anti-LN antibody as the probe. LEW rats [Harlan Sprague–Dawley; Indianapolis, IN, (RT1^{l})], thymectomized at 7 weeks of age, X-radiated (750 rads) and bone marrow reconstituted 4 weeks later, are severely T-cell deficient and unable to reject major histocompatibility complex (MHC) incompatible (L × BN)F$_1$ (RT1$^{l/n}$) cardiac allografts.[9] T-cell deficient engrafted hosts were used in these experiments for two reasons: (i) because the control of lymphocyte migration *in vivo* still constitutes a fundamental challenge to the success of organ transplantation; and (ii) because putative LN binding sites in lymph node stroma could be fully occupied in animals with their full quota of circulating lymphocytes, and thus thwart the 'visibility' of an experimental effect of the anti-LN antibodies.

Abbreviations: ECM, extra cellular matrix; HEV, high endothelium venules; LN, laminin; PLN, peripheral lymph node; PCV, post-capillary venules.

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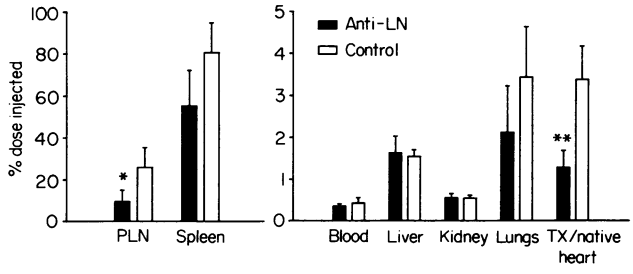

Figure 1. Migration patterns (percentage dose injected/g tissue) of ^{111}In-labelled PLN cells in B recipients of cardiac allografts pretreated with rabbit anti-LA or rabbit serum (control). T-cell deficient LEW recipients of $(L \times BN)F_1$ cardiac allografts were treated with rabbit anti-LN antibody ($n = 6$) or rabbit serum ($n = 3$). Thirty minutes later the animals received ^{111}In-labelled syngeneic PLN cells. The rats were killed 6 hr after cell transfer, their tissues weighed and radioactivity measured. Means and SD are shown. Statistical significance of differences between anti-LN and control groups was ascertained by Student's *t*-test: $*P < 0{\cdot}05$; $**P < 0{\cdot}001$.

Planning the experiment not knowing the biodistribution of antibody after i.v. injection, we reasoned that the antibody should be given shortly before the transfer of labelled cells (30 min) and the animals killed at a time sufficiently late for the circulation of transferred cells to be stabilized and sufficiently early for an effect to be seen. One hour was thought to be too early; 24 hr too late. The 6-hr migration patterns following an i.v. injection of ^{111}I-oxyquinoline (Amersham Corp., Arlington Heights, IL; 37MBq/ml)-labelled PLN cells ($20–30 \times 10^6$, 2–3 μCi/10^8) from LEW rats were compared in syngeneic B recipients of $(L \times BN)F_1$ cardiac allografts, pretreated with a rabbit anti-rat LN antibody (200 μg of protein; Chemicon Internat. Inc., Temecula, CA) or rabbit serum. The biodistribution of the ^{111}In cells was determined in the following compartments: peripheral blood, PLN, spleen, kidney, liver, lung, grafted and native hearts. The summary of the results, expressed as percentage of injected radioactivity per gram of tissue is shown in Fig. 1. The accumulation of adoptively transferred cells was significantly reduced in the PLN of the anti-LN-treated rats compared to controls ($9{\cdot}63 \pm 5{\cdot}5$ versus $25{\cdot}9 \pm 9{\cdot}5$, $P < 0{\cdot}05$). Although higher numbers of cells were recovered also in control ($80{\cdot}6 \pm 14{\cdot}2$) than in the anti-LN spleens ($55{\cdot}4 \pm 16{\cdot}7$), this difference was not statistically significant. Similarly, lungs of control recipients collected slightly more cells ($3{\cdot}43 \pm 1{\cdot}2$) than those in anti-LN modulated hosts ($2{\cdot}1 \pm 1{\cdot}1$, NS). The most striking differences, however, were observed in the amount of radioactivity recovered from the allografts in both animal groups. The results, expressed in terms of grafted : native heart activity ratios, show that the mean ratio in the anti-LN-treated recipients ($1{\cdot}28 \pm 0{\cdot}4$) was significantly lower compared to that in the rabbit serum-treated controls ($3{\cdot}37 \pm 0{\cdot}8$, $P < 0{\cdot}001$). In contrast, no differences were seen in liver, blood or kidney.

To our knowledge, this work provides the first direct evidence that LN could act as an ECM component 'signal' for lymphocyte migration to specific organs, including an allograft. It has been shown that human melanoma cells express integrin receptors for LN[10] and that LN influences *in vivo* tumour cell migration by a mechanism that may involve their increased attachment to collagen type IV (reviewed by Hunt, 1989).[11] Together, the present results confirm the much earlier conclusion that 'adhesive interactions between circulating and resident cells seem to play an important role in the control of metastatic spread' and of lymphocyte traffic.[3]

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