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Adhesion molecule expression trigger immune-mediated pathology in lupus-nephritis

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects many organs including the joints, kidneys and brain. Lupus nephritis is a potentially devastating complication of SLE. Immune cells, cytokines, and epigenetic factors have all been recently implicated in lupus nephritis pathogenesis. We have hypothesized that their Adhesion molecule expression trigger immune-mediated pathology in lupus-nephritis. We used female MRL/lpr, MRL/mpJ (develops lupus-like disease at 10-14 months of age) and C3H/HeJ mice. Using immunofluorescent antibody binding assays and confocal laser imaging, we show that expression of ICAM-1 and VCAM-1 is elevated in MRL/lpr kidney at 3-6 months of age as compared to age-matched controls. These results suggest a possible mechanism for leukocyte entry into the kidney of autoimmune mice that in turn suggest immune-mediated pathology in renal-lupus and may enable a paradigm shift in the treatment of this complex disease.

Keywords: SLE; Adhesion molecule expression; cytokines; ICAM-1; VCAM-1

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Introduction

Systemic lupus erythematosus (SLE) is a highly complex and heterogeneous autoimmune condition that mainly affects women in their reproductive years. Despite the advent of disease classification, monitoring strategy and therapeutic options, survival of SLE patients has not improved much further since the 1980s [1]. The heterogeneous nature of the disease and the lack of full understanding of the pathogenic mechanism are major hurdles to further improving the current management strategy of SLE [2].

Lupus nephritis affects up to 70% of patients with systemic lupus erythematosus and is an important treatable cause of kidney failure. Cardinal features of lupus nephritis include loss of self-tolerance, production of autoantibodies, immune complex deposition and immune-mediated injury to the kidney, resulting in increased cell proliferation, apoptosis, and induction of inflammatory and fibrotic processes that destroy normal nephrons [3]. Is an important cause of kidney failure in patients of Asian, African, or Hispanic descent. Its etiology and pathogenesis

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are multifactorial and remain to be elucidated [4]. Accumulating evidence suggests that anti-double-stranded DNA (dsDNA) antibodies play a critical role in the pathogenesis, through its direct binding to cross-reactive antigens on resident renal cells or indirect binding through chromatin material to extracellular matrix components, resulting in complement activation, cell activation and proliferation, and induction of inflammatory and fibrotic processes.

SLE is a chronic inflammatory disease that can affect any organ, but very often injures the kidney. SLE is more prevalent in women than men across all age groups and populations; the female-to-male ratio is highest at reproductive age, ranging between 8:1 and 15:1, and is lowest in prepubertal children at about 4:3 [5].

The prevalence of SLE and the chances of developing lupus nephritis (LN) vary considerably between different regions of the world and different races and ethnicities [6). In the United States, the higher frequency of LN in black populations persists after adjustment for socioeconomic factors [7]. Additionally, black and Hispanic SLE patients develop LN earlier [8], and have worse outcomes than white patients with SLE, including death and ESRD [1]. This might explain why black individuals account for nearly half of those with ESRD due to LN, the more aggressive disease course in black individuals might be the result of a higher incidence of diffuse proliferative LN, or the presence of more high-risk features within the same LN histologic class when compared with white individuals [9].

Those differences may arise due to genetic predisposition as some “high-risk” genotypes and autoantibodies are more frequent in black patients [10]. For example, black populations have a higher frequency of the *Fcγ RIIA*-R131 allele which is involved in mediating phagocytosis of IgG2 immune complexes [11]. The APOL1 gene, which has been implicated in the development of ESRD in black patients, has also been associated with progression and development of ESRD in the LN population [12].

In LN patients with two risk alleles for APOL1 the odds ratio (OR) for ESRD was 2.72 (95% confidence interval [95% CI], 1.76 to 4.19; $P<6.2\times10^{-6}$). An HLA-DR2 subtype (HLA-DRB181503), characteristic of black populations, was linked to worsening proteinuria [13]. Black individuals are also more likely to have positive anti-Ro, anti-Sm, and anti-RNP antibodies, which have a high association with LN [14].

Materials and Methods

Animals

Female MRL/lpr, MRL/mpJ (develops lupus-like disease at 10-14 months of age) and C3H/HeJ mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). ICAM-1$^{-/-}$ MRL/mpJ-mice were generated by back-crossing a gene-targeted mutation on to the MRL/MpJ strain background, as described previously [15]. Mice were used at 10-14 months of age and weighed 30–50 g.

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Immunohistochemistry and histological evaluation

After deparaffinization and rehydration, paraffin sections were blocked with Dual Endogenous Enzyme Block (DAKO, Glostrup, Denmark) for immunohistochemistry. The primary antibody was rabbit anti-CD3 (or B220, Iba-1, Ki-67, TGF-β1, PDGF-B, CTGF, α-smooth muscle actin [SMA], fibronectin, collagen I) IgG (Abcam, Cambridge, MA). The secondary antibody was polymer-horseradish peroxidase-labeled goat anti-rabbit IgG (DAKO). The detection of glomerular IgG deposition was performed with horseradish peroxidase-labeled goat anti-mouse IgM, IgG, IgG1, IgG2a, IgG2b, and IgG3 (Thermo Fisher Scientific) as described previously [8]. Finally, sections were incubated with 3, 3′-diaminobenzine-chromogen substrate (DAKO) and counterstained with hematoxylin. The stains were scored by a renal pathologist blinded to the mice grouping.

ELISA

ELISAs were performed with serum or urine according to the manufacturer's instructions. Immunoassay kits (targeting mouse TGF-β1 and platelet-derived growth factor B [PDGF-B]) were purchased from R&D Systems, and the connective tissue growth factor (CTGF) kit was from MyBiosource Inc. (San Diego, CA). Mouse microalbuminuria kit was purchased from Elabscience (China). Total anti-dsDNA IgG and IgG isotype concentrations were measured by ELISA in serum diluted 1:200 as described [5]. The optical density values were read at 450 nm.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from fresh tissue by Trizol reagent (Ambion, Carlsbad, CA). Complementary DNA was generated with a commercial kit (Takara Bio, Kyoto, Japan). qRT-PCR was performed in triplicate with TB green stain (Takara Bio) and the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Waltham, MA). The expression levels of the objective genes were calculated with the $2^{-\Delta Ct}$ method.

Statistical Analysis

All data were expressed as mean ± standard error of mean. GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA) was used for statistical analysis. Analysis of variance (ANOVA) was used for the comparison of more than two groups. Then, the differences between two groups were compared by the two-tailed Student *t* test. Differences were considered to be statistically significant at $p < 0.05$.

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Results

Comparison of the phenotypes of circulating leucocytes in MRL$^{+/+}$ and MRL/MpJ-lpr mice revealed that at 8 weeks, the numbers of neutrophils, monocytes, CD4 and CD8 T cells and B cells were significantly lower in MRL/MpJ- lpr mice (Tables 1). In MRL$^{+/+}$ mice, leucocyte counts remained relatively stable to 10 months. In contrast, in MRL/MpJ lpr mice at 14 months, there was a marked increase in the number of circulating leucocytes. Much of this change was due to a striking elevation in circulating T cells of almost 10 × 10^6/ml, with an increase in circulating DN T cells accounting for the majority of this difference. In MRL$^{+/+}$ mice, DN T cells represented a very minor population. Small but significant increases in neutrophils and monocytes were also observed in 16-week MRL/MpJ- lpr mice. By 20 weeks, the leucocyte counts in MRL/MpJ- lpr mice had undergone a further increase to nearly 30 × 10^6/ml, with more than 14 × 10^6/ml being DN T cells.

Effect of ICAM-1 deficiency on infiltration

We next examined leucocyte infiltration in MRL/MpJ-lpr mice deficient in either ICAM-1 or P-selectin. In 10-months-old mice, neither adhesion molecule deficiency resulted in a reduction in choroid leucocyte infiltration (Fig. 1). In contrast, ICAM-1$^{-/-}$ MRL/MpJ-lpr mice displayed increased leucocyte recruitment, apparent as a significant increase in the density of leucocyte infiltrate within the affected area did not differ from wild-type mice.

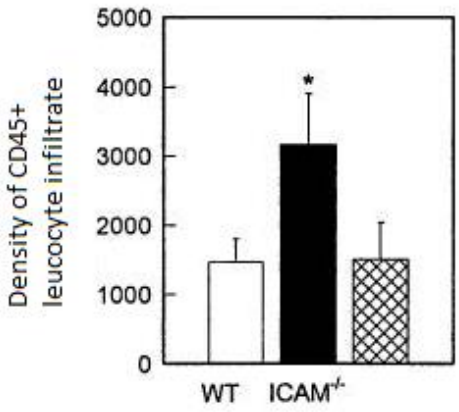

Figure 1.

Density of CD45$^+$ leucocyte infiltrate present in the renal of each of the mouse strains. *n* = 7 for WT mice and 4 for ICAM-1$^{-/-}$. Data are shown as ± s.e.m. **P* < 0·05 *versus* MRL/MpJ-lpr mice.

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Effect of ICAM-1

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Assessment of the types of leucocytes present in the renal of these mice showed that at 14 months, a greater proportion of both T and B cells were present in brains of both ICAM-$1^{-/-}$and MRL/MpJ-lpr mice, relative to wild-type MRL/MpJ-lpr mice. At 10 months, ICAM-$1^{-/-}$ mice had a significantly increased proportion of $CD4^+$ cells, whereas at 14 months, DN T cells were significantly increased in MRL/MpJ-lprmice, relative to wild-type MRL/MpJ-lpr mice.

Role of ICAM-1 on phenotype of circulating leucocytes in MRL/MpJ-lpr mice

Mice deficient in ICAM-1 on conventional backgrounds have been described previously to have increased circulating leucocyte counts [16]. Therefore, we examined circulating leucocyte phenotypes of these mice on the MRL/MpJ-lpr background (Tables1). At 10 months, MRL/MpJ-lpr mice displayed significant elevations in all cell types, but most prominently in neutrophils. At 10 and 14 months, the number of circulating leucocytes in both strains showed comparable elevations (relative to $MRL^{+/+}$ mice) to those seen in wild-type MRL/MpJ-lpr mice. However, ICAM-$1^{-/-}$ mice showed evidence of a delay in the increase in $CD3^+$ cells, as at 10 months the number of $CD3^+$ cells was lower than that seen in wild-type MRL/MpJ-lpr mice, due to reduced numbers of CD8 and DN T cells. However, by 14 months, ICAM-$1^{-/-}$mice had become similar to wild-type MRL/MpJ-lpr mice, apart from an alteration in the proportions of T cells such that CD4 cells were increased significantly and DN T cells were decreased significantly (Tables 1).

Histological examination

Leucocyte infiltration in renal of MRL/MpJ-lpr mice revealed the cortex was the primary site of leucocyte infiltration (Fig. 1), with leucocytes rarely detectable within the parenchyma [10]. Therefore, detailed comparison of $MRL^{+/+}$ and MRL/MpJ-lpr mice was based on analysis of the cortex. In $MRL^{+/+}$ mice, that contained diffuse areas of $CD45^+$ cell infiltration (Fig. 2a). In contrast, the intensity of infiltration in MRL/MpJ-lpr mice was such that the size of the cortex appeared increased relative to $MRL^{+/+}$ mice (Fig. 2b). We therefore quantified the size and intensity of infiltration in $MRL^{+/+}$ and MRL/MpJ-lpr mice. At 14 months, the mean area in MRL/MpJ-lpr mice was greater than twice that in $MRL^{+/+}$ mice ($P< 0{\cdot}05$) (Fig. 2a).

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Table 1.

Alteration in the proportions of T cells such that CD4 cells were increased significantly and DN T cells were decreased significantly.

	Total	M1/70^{hi}/Gr-1^{hi} (PMNs)	M1/70 ^{hi}/Gr-1^{int} (monocyte lineage)	CD3^{+} (T cells)	B220^{+}/CD3^{-} (B cells)
MRL$^{+/+}$					
8 weeks	6·8 ± 0·5 (6)	1·5 ± 0·1 (6)	0·20 ± 0·28 (6)	3·2 ± 0·3 (6)	2·1 ± 0·2 (6)
16 weeks	7·4 ± 0·5 (6)	1·6 ± 0·1 (6)	0·20 ± 0·01 (6)	3·2 ± 0·2 (6)	1·7 ± 0·1 (6)
20–25 weeks	4·9 ± 0·6 (7)	1·3 ± 0·2 (7)	0·14 ± 0·05 (7)	1·9 ± 0·2 (7)	0·9 ± 0·2 (7)
MRL/MpJ-lpr					
8 weeks	5·3 ± 0·4 (6)	0·9 ± 0·1 (6)†	0·07 ± 0·01 (6)†	2·0 ± 0·1 (9)†	1·0 ± 0·1 (9)†
16 weeks	19·4 ± 3·8 (6)	4·5 ± 0·9 (6)†	0·77 ± 0·17 (6)†	12·8 ± 1·3 (6)†	1·4 ± 0·1 (6)
20–25 weeks	29·6 ± 4·1 (7)	5·3 ± 1·8 (6)†	0·72 ± 0·18 (6)†	20·8 ± 1·6 (6)†	2·0 ± 0·2 (6)†
MRL/MpJ-lpr					
8 weeks	10·9 ± 0·5 (9)	4·4 ± 0·3 (8)**	0·56 ± 0·07 (8)**	3·1 ± 0·4 (6)**	1·7 ± 0·3 (6)**
16 weeks	20·2 ± 4·9 (5)	5·7 ± 0·7 (6)	0·79 ± 0·14 (6)	10·7 ± 0·8 (8)	2·8 ± 0·2 (8)**
20–25 weeks	27·8 ± 4·9 (6)	6·5 ± 1·6 (6)	0·72 ± 0·16 (6)	14·6 ± 1·6 (7)**	3·1 ± 0·4 (7)**
ICAM-1$^{-/-}$-MRL/MpJ-lpr					
16 weeks	16·1 ± 4·4 (5)	3·2 ± 0·8 (6)	0·50 ± 0·13 (6)	7·3 ± 0·7 (8)**	2·0 ± 0·2 (8)**
20–25 weeks	28·7 ± 6·7 (5)	4·3 ± 0·8 (6)	0·51 ± 0·09 (6)	18·1 ± 0·8 (6)	4·7 ± 0·7 (6)**

*Data are expressed as cells × 10^6/ml ± s.e.m. The number of mice sampled is shown in parentheses.
†*P* < 0·05 *versus* MRL$^{+/+}$ at same age.
**P < 0·05 *versus* MRL/MpJ-lpr at same age.

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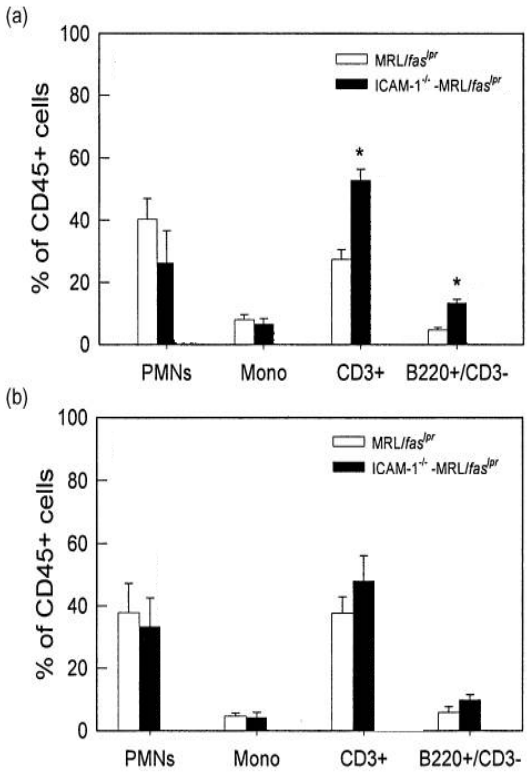

Figure 2.

Leucocyte infiltration in renal of MRL/MpJ-lpr mice

Discussion

Deficiency in ICAM-1 has been shown previously to have a specific effect on neutrophil migration, as shown by a fourfold increase in circulating neutrophils and a concomitant decrease in neutrophil migration to sites of inflammation [17].

This suggests that alternative adhesion molecules may assume the roles played by ICAM-1 in supporting leucocyte entry into the brain. This possibility is supported by the fact that the inflammation which affects the MRL/MpJ-lpr mouse occurs over several months, allowing time for expression of alternative adhesion molecule pathways.

The reason the renal is a preferential target of eucocyte recruitment in MRL/MpJ-lpr mice is unclear. It is possible that the fenestrated endothelium of the ortex allows leucocyte entry to occur more readily, and the preferential deposition of immune complexes in this site may also act to attract and retain leucocytes [18]. In addition, investigation of adhesion molecule expression in the choroid has demonstrated that the choroid epithelial cells, but not the associated endothelial cells, constitutively express ICAM-1 and VCAM-1, and increase expression of these molecules during inflammatory responses [19]. This finding raises the possibility that following leucocyte exit from the vasculature, epithelial cell-expressed molecules

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are important in retaining leucocytes at this site. It is clear from the present findings that ICAM-1 does not serve this function, as large numbers of leucocytes accumulate in the cortex of ICAM-1$^{-/-}$ MRL/MpJ-lpr mice. However, epithelial cell-expressed VCAM-1 could act in this fashion as many of the leucocyte types present in the choroid, including DN T cells, express VCAM-1 ligands such as the α4-integrin, making them capable of adhering to VCAM-1-expressing cells [19-22].

Recent observations have shown that in some tissues the necessity for a rolling interaction prior to leucocyte adhesion to the endothelium is bypassed [23, 25]. Furthermore, in renal microvessels, adhesion of activated lymphoblasts has been observed to occur without prior rolling [24]. Given these findings, it is conceivable that in the cortex vasculature the conventional multi-step paradigm of leucocyte recruitment does not apply. Under these circumstances it is plausible that ICAM-1 is required for leucocyte adhesion within the cortex vasculature. Further experiments with MRL/MpJ-lpr mice lacking alternative adhesion molecules would aid in clarifying this issue.

Conclusions

Our study demonstrated that ICAM-1 ameliorates the murine model of LN, possibly through inhibiting renal cytokines and chemokines and relevant tissue inflammation and fibrosis.

Author Contributions

JB, SC, SD wrote the paper. RT, MS, RW, YW, PF design the study and provided substantial revision. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Rekvig OP, Putterman C, Casu C, et al. Autoantibodies in lupus: culprits or passive bystanders? Autoimmun Rev 2012;11(8):596-603. [PubMed]
2. Bae SC, Koh HK, Chang DK, et al. Reliability and validity of Systemic Lupus Activity Measure-Revised (SLAM-R) for measuring clinical disease activity in systemic lupus erythematosus. Lupus 2001;10:405–9. [PubMed]
3. Couser WC, Salant DJ, Madaio MP, et al. Factors influencing glomerular and tubulointerstitial patterns of injury in SLE. Am J Kidney Dis 1982;2:126–134. [PubMed]
4. Underhill GH, Minges Wols HA, Fornek JL, et al. IgG plasma cells display a unique spectrum of leukocyte adhesion and homing molecules. Blood 2002;99:2905–2912. [PubMed]
5. Takahashi S, Araki K, Araki M, et al. Suppression of experimental lupus nephritis by aberrant expression of the soluble E-selectin gene. Pathol Int 2002;52:175–180. [PubMed]
6. Sires RL, Adler SG, Louie JS, Cohen AH. Poor prognosis in end-stage lupus nephritis due to nonautologous vascular access site associated septicemia and lupus flares. Am J Nephrol 1989;9(4):279-84. [PubMed]
7. Chaussabel D, Quinn C, Shen J, et al. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. Immunity 2008;29:150–64. [PubMed]
8. Zameer A, Hoffman T. Increased ICAM-1 and VCAM-1 expression in the brains of autoimmune mice. Journal of Neuroimmunology 2003;142:67-74. [Abstract/FullText]
9. Seelen MA, Trouw LA, Daha MR. Diagnostic and prognostic significance of anti-C1q antibodies in systemic lupus erythematosus. Curr Opin Nephrol Hypertens 2003;12(6):619-24. [PubMed]
10. Vasudevan A, Krishnamurthy AN. Changing worldwide epidemiology of systemic lupus erythematosus. Rheum Dis Clin North Am 2010;36(1):1-13. [PubMed]
11. Deapen D, Escalante A, Weinrib L, et al. A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum 1992;35:311–318. [PubMed]
12. McCarty DJ, Manzi S, Medsger TA Jr, et al. Incidence of systemic lupus erythematosus. Race and gender differences. Arthritis Rheum 1995;38:1260–1270. [PubMed]
13. Dai Y, Sui W, Lan H, et al. Comprehensive analysis of microRNA expression patterns in renal biopsies of lupus nephritis patients. Rheumatol Int 2009;29:749–754. [PubMed]
14. Dai Y, Huang YS, Tang M, et al. Microarray analysis of microRNA expression in peripheral blood cells of systemic lupus erythematosus patients. Lupus 2007;16:939–946. [PubMed]
15. Schiffer L, Kumpers P, Davalos-Misslitz AM, et al. B-cell-attracting chemokine CXCL13 as a marker of disease activity and renal involvement in systemic lupus erythematosus (SLE). Nephrol Dial Transplant 2009;24:3708–3712. [PubMed]
16. Peterson KS, Huang JF, Zhu J, et al. Characterization of heterogeneity in the molecular pathogenesis of lupus nephritis from transcriptional profiles of laser-captured glomeruli. J Clin Invest 2004;113:1722–1733. [PubMed]
17. Davies MJ, Gordon JL, Gearing AJ, et al. The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. J Pathol 1993;171:223229. [PubMed]
18. Villanueva E, Yalavarthi S, Berthier CC, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol 2011;187:538–552. [PubMed]
19. Hill GS, Delahousse M, Nochy D, et al. Predictive power of the second renal biopsy in lupus nephritis: significance of macrophages. Kidney Int 2001;59:304–316. [PubMed]
20. Fismen S, Mortensen ES, Rekvig OP. Nuclease deficiencies promote end-stage lupus nephritis but not nephritogenic autoimmunity in (NZB x NZW) F1 mice. Immunol Cell Biol 2011;89:90–99. [PubMed]
21. Collins RG, Velji R, Guevara NV, et al. P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice. J Exp Med 2000;191:189–194. [PubMed]
22. Jung GS, Kim MK, Jung YA, et al. Clusterin attenuates the development of renal fibrosis. J Am Soc Nephrol 2002;23:73–85. [PubMed]

Research Article http://dx.doi.org/10.18081/ajbm/2333-5106-013-12/49-57

23. Torres R, de Castellarnau C, Ferrer LL, et al. Mast cells induce upregulation of P-selectin and intercellular adhesion molecule 1 on carotid endothelial cells in a new in vitro model of mast cell to endothelial cell communication. Immunol Cell Biol 2002;80:170–177. [PubMed]
24. Tidball JG. Inflammatory processes in muscle injury and repair. Am J Physiol Regul Integr Comp Physiol 2005;288:R345–353. [PubMed]
25. W G James 1, P Hutchinson, D C Bullard, M J Hickey. Cerebral leucocyte infiltration in lupus-prone MRL/MpJ-fas lpr mice--roles of intercellular adhesion molecule-1 and P-selectin. Clin Exp Immunol 2006;144(2):299-308.

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