SCIENTIFIC REPORT

Susceptibility to endotoxin induced uveitis is not reduced in mice deficient in BLT1, the high affinity leukotriene B₄ receptor

J R Smith, K Subbarao, D T Franc, B Haribabu, J T Rosenbaum

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Aim: To investigate the role of arachidonic acid derived chemotactic factor, LTB₄, in the development of endotoxin induced uveitis (EIU), using mice deficient in the BLT1 gene which encodes the high affinity LTB₄ receptor.

Methods: BLT1 gene deficient and wild type BALB/c mice were injected intravitreally with *Escherichia coli* 055:B5 lipopolysaccharide (250 ng/2 µl). Number of leukocytes invading the anterior chamber 24 hours later were counted on tissue cross sections.

Results: In all mice, EIU was characterised by a polymorphonuclear and mononuclear cell infiltrate. Numbers of infiltrating cells did not differ significantly between control and BLT1 gene knockout mice.

Conclusion: Chemotactic factors other than LTB₄ are primarily responsible for leukocyte migration into the eye during murine EIU.

Endotoxin induced uveitis (EIU) is a well characterised experimental model of acute uveal inflammation that may be induced in several species by systemic or intraocular injection of lipopolysaccharide.^{1–3} The inflammation is characterised by infiltration of the iris and ciliary body by leukocytes, the majority of which are neutrophils and monocytes/macrophages, followed by cellular exudation into the anterior chamber.¹ Although a substantial number of the molecular mediators of EIU have been identified, the exact roles and relative importance of many of these signals have still to be clarified.⁴ Leukotriene B₄ (LTB₄), an arachidonic acid metabolite that is strongly chemotactic for neutrophils and monocytes/macrophages,⁵ has been implicated as an initiator of EIU in studies using rats.⁶ However, the role of LTB₄ in EIU, as well as other forms of experimental uveal inflammation, and in human uveitis is still debated.⁷ One of the authors (BH) has used gene targeting in embryonic stem cells to disrupt the murine BLT1 gene, which encodes the high affinity LTB₄ receptor mediating inflammatory responses in the mouse.⁸ These BLT1 gene deficient animals have provided us with an opportunity to study whether and in what capacity LTB₄ is required for the development of EIU.

MATERIALS AND METHODS

BLT1 gene deficient mice were generated on a C57BL/6×129 SvJ background, as previously described,⁸ and subsequently back crossed on to a BALB/c background over seven generations. Along with wild type BALB/c mice, the gene knockout mice were used at 12 weeks of age or less. In a single experiment, male and female animals, anaesthetised by inhalational isoflurane (Abbott Laboratories, North Chicago, IL, USA), were injected intravitreally with

Escherichia coli 055:B5 lipopolysaccharide (Sigma Chemical Co, St Louis, MO, USA) reconstituted in phosphate buffered saline (250 ng/2 µl). Animals were killed 24 hours after injection, and enucleated right eyes fixed in 10% buffered formalin for 24 hours, then transferred to 70% ethanol for a minimum of 24 hours, prior to embedding in paraffin. Tissue cross sections through the optic nerve were cut 5 µm thick and stained with haematoxylin and eosin. Numbers of leukocytes that were present in the anterior chamber were counted under light microscopy by a masked investigator. Although inflammatory cells were also observed in the posterior segment of the eyes, these cells were not included in the count, due to the possibility that injection related trauma might influence the number. Total number of anterior chamber cells for BLT1 gene deficient mice and wild type control animals were compared by the Mann-Whitney U test.

RESULTS

As illustrated in figure 1, in both wild type and BLT1 gene deficient BALB/c mice, EIU was characterised by a polymorphonuclear and mononuclear cell infiltration of the anterior segment of the eye. Figure 2 shows that there was no significant difference in the number of cells counted in the anterior chamber between controls (n = 16 mice, median number of inflammatory cells = 82, range 0–314) and gene knockout mice (n = 13 mice, median number of inflammatory cells = 88, range 2–505) (p = 0.39). There was also no significant difference in anterior chamber cell counts obtained for BLT1 gene deficient male versus female animals (data not shown).

CONCLUSION

Although BLT1 gene deficient mice demonstrate reduced neutrophil and monocyte/macrophage influx in a zymosan induced model of peritonitis,⁸ these gene knockout mice develop EIU with a pattern and severity that is identical to inflammation observed in wild type controls. The severity of EIU is highly strain dependent. Accordingly, interpretation of some other studies of EIU using gene knockout mice is complicated by genetic variation between animals related to the crossed C57BL/6×129 background strain.⁹ One strength of this study is that the BLT1 gene deficiency has been back crossed on to a single background strain. Our findings suggest that BLT1 is not a critical mediator of murine EIU, and question the contribution of LTB₄ to leukocyte extravasation in this experimental model.

Abbreviations: EIU, endotoxin induced uveitis; LTB₄, leukotriene B₄

Figure 1 In both wild type (A) and BLT1 gene deficient (B) BALB/c mice, endotoxin induced uveitis was characterised by a polymorphonuclear and mononuclear infiltrate in the anterior segment of the eye (stain: haematoxylin and eosin; original magnification, $\times 400$).

While it is possible that LTB₄ acts via a second receptor—BLT2—in EIU, this is unlikely. Expressed predominantly on certain lymphocyte subsets and monocytes, BLT2 is a relatively low affinity LTB₄ receptor.¹⁰ The role of this receptor in LTB₄ mediated inflammatory responses remains to be determined, but its presence does not compensate for absence of BLT1 in other murine models of inflammation.⁸

Additional chemotactic factors must be primarily responsible for leukocyte migration from the vascular tree into the anterior uveal tissue during the initiation phase of EIU. Indeed, our own previous studies in rabbits demonstrated that intravitreal injection of lipopolysaccharide consistently increased aqueous levels of LTB₄, but the increase accounted at most for a minority of the chemotactic activity present in this aqueous.² Results of another study from our group, in which EIU was induced in interleukin (IL)-8 receptor homologue gene deficient and wild type mice, contrast with the findings of the present report, and implicate chemoattractants that bind the IL-8 receptor as critical in this model.³ Among IL-8 receptor homologue knockout mice, there was a highly statistically significant reduction in the number of cells that infiltrated the iris in response to intravitreal lipopolysaccharide injection, although rolling and sticking within the local vessels were unaffected. Bhattacharjee and Henderson have provided evidence that IL-1, which rises to high levels within the eye during EIU, may also be a more important chemotactic signal than LTB₄ in EIU.¹¹ In rabbits, anterior chamber injection of IL-1 resulted in a substantially higher number of accumulated aqueous cells than did a similar injection of LTB₄.

Results from a study in humans corroborate our observations.¹² Analysis of aqueous fluid taken from patients with uveitis showed no significant elevation in the level of LTB₄ over levels measured in controls with no history of

Figure 2 There was no significant difference ($p=0.39$) in the total number of inflammatory cells infiltrating the anterior chamber of wild type and BLT1 gene deficient BALB/c mice 24 hours following intravitreal injection of 250 ng of lipopolysaccharide. Total number of animals in each group indicated by n. One triangle represents one animal, and bars indicate the median cell count per group.

uveitis, whose aqueous was sampled during cataract extraction or penetrating keratoplasty. Additionally, the level of LTB₄ did not correlate with the aqueous cell count made by slit lamp examination, a recognised index of severity of uveitis. Given our results and current literature, we suggest that a treatment targeting the high affinity LTB₄ receptor, BLT1, alone is unlikely to significantly influence the course of murine EIU, and question the clinical utility of such a therapeutic approach for forms of human uveitis that involve similar pathogenic mechanisms.

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Authors' affiliations

J R Smith, D T Franc, J T Rosenbaum, Casey Eye Institute, Oregon Health & Science University, Portland, OR, USA

K Subbarao, B Haribabu, James Graham Brown Cancer Center and Department of Microbiology & Immunology, University of Louisville, Louisville, KY, USA

Correspondence to: Dr J R Smith, Casey Eye Institute, Oregon Health & Science University, 3375 SW Terwilliger Blvd, Portland, Oregon, USA 97239-4197; smithjrs@ohsu.edu

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