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Intestinal reperfusion injury is mediated by IgM and complement

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Keywords

immunoglobulin M, rodent, inflammation, complement, transgenic, knockout

Comments

This study, through genetically modified mice strains, manages to confirm the findings of previous studies; that complement is important in ischemia-reperfusion injury, and further defines the classical pathway as important via IgM binding. However, it does leave many questions yet to be answered, including the trigger for IgM binding. Level V study.

Introduction

The mechanism of injury resulting from reperfusion after ischemia involves inflammatory mediators (including the complement system and cytokines). The degree of injury is related to the duration of ischemia, the size of ischemic area and the particular tissue involved. With small intestine, 45 min of ischemia produces mucosal destruction but has little effect on the surrounding smooth muscle.

From intestinal ischemia studies involving rats treated with soluble complement receptor inhibitor (sCRI), it has been shown that reperfusion injury is related to the activation of complement. In mice studies, it has also been shown that mice genetically deficient in C4, C3 or immunoglobulins were protected from muscle injury. However, it has been unclear whether intestinal reperfusion injury was produced by the classical or alternative pathway of complement activation.

Aims

Using mice strains deficient in C3 or C4 or immunoglobulins, the relative roles of these factors were examined in a jejunal ischemia-reperfusion murine model.

Methods

Mice strains used were either:

1) C4 deficient (serum C4 undetectable with ELISA)
2) C3 deficient (serum C3 undetectable with ELISA)
3) Totally deficient in immunoglobulins but normal complement components
4) Wild type

All mice were anesthetized with intraperitoneal pentobarbitol and had a laparotomy. A tourniquet was applied to the most proximal 6 cm of jejunum to occlude the mesenteric vessels and jejunal lumen. The tourniquet was applied for 40 min then removed and reperfusion checked by ensuring return of pulsatile flow to mesenteric arcade. Five min before reperfusion, all animals were given ^{125}iodine labelled bovine serum albumin. Wild type mice received sCRI, which is a complement antagonist. Some group 3 mice (deficient in immunoglobulins) were given IgM intravenously 30 min before initial laparotomy.

At the end of 3 h reperfusion, the jejunum was harvested and counted for radioactivity and the intestinal permeability index (PI) determined. Immunohistological analysis for IgM, IgG and C3 was also performed. Sham animals had a laparotomy but no induced intestinal ischemia.

Results

1) The classical complement pathway mediates reperfusion edema

Wild type mice (n = 12) had higher PI compared to sham (n =5). The PI for C3 deficient mice (n= 8) was lower than wild type. Therefore, deficiency in C3 (impairing classical and alternative pathways) resulted in 40% reduction in permeability.

Wild type mice given sCRI (n= 5) had a 60% reduction in permeability. sCRI binds and degrades both C3b and C4b so inhibiting the alternative as well as the classical pathway. C4 deficient mice (intact alternative pathway but cannot form classical pathway C3 convertase C2aC4b) had a 42% reduction in permeability, which was similar to that for C3 deficient mice. Therefore, the permeability increase seen following ischemia-perfusion injury, is by activation of the classical pathway, not the alternative pathway.

2) Classical complement activation is dependent on IgM

Group 3 mice (n = 4), with normal complement but deficient in IgM and IgG, were also protected with a 75% reduction in permeability. When some of these mice (n = 5) were given IgM, they had similar PI to control mice. Immunohistochemical analysis showed IgM deposited in reperfused mice (but not sham mice). C3 was colocated with IgM on the mucosa of reperfused wild type mice. C4 and C3 deficient mice showed less staining for IgM and minimal staining for C3 compared to wild type mice. Staining for IgG deposit was minimal in all groups.

Discussion

Intestinal ischemia-reperfusion injury is dependent on complement, and injury reduction is seen in animals treated with sCRI. This has been shown in other tissues in previous studies. C4 deficient mice are protected from injury to a similar degree as C3 deficient mice, and this indicates that the classical pathway of complement activation mediates injury. IgM and not IgG is localised to reperfused endothelium. Immunoglobulin deficient animals given IgM, restored permeability changes to levels seen in wild type mice, indicating that IgM fixation is responsible for complement activation leading to jejunal injury and increased vascular permeability. The mechanisms by which reperfused tissue acquires IgM binding capacity and complement deposits, are not known.

References

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