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Decay-accelerating factor attenuates remote ischemia–reperfusion-initiated organ damage ☆

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Abstract Complement activation contributes to the expression of local and remote organ injury in animal models of ischemia–reperfusion (IR). We demonstrate here that a soluble form of decay-accelerating factor (DAF) protects normal C57Bl/6 and autoimmunity-prone B6.MRL/*lpr* mice subjected to hindlimb IR from remote intestinal and lung injury without affecting the degree of local skeletal muscle injury. In addition, DAF treatment attenuates remote organ injury in mice subjected to mesenteric IR. Soluble DAF allowed the deposition of complement 3 in local and remote injury sites while it limited the presence of terminal membrane attack complex and did not increase animal susceptibility to sepsis. These data provide evidence that soluble DAF might offer clinical benefit to patients suffering remote intestinal or lung damage in response to muscle or other organ injury.

Introduction

Ischemia–reperfusion (IR) is a common clinical entity encountered in diverse fields such as vascular surgery, trauma, cardiac surgery, transplant, and cardiovascular medicine. The acute inflammatory response following an ischemic insult instigates pathology in local and remote organs leading to significant morbidity and mortality. The critical role of complement activation in tissue injury has been amply demonstrated, resulting in various attempts at therapeutic intervention.

Observations of decreased myocardial inflammation with transient depletion of complement (C) 3 [1] and of the beneficial effect of soluble complement receptor 1 (sCR1) on IR pathology [2–7] implicated a role for complement in IR tissue damage. Studies in *C3*- and *C4*-deficient mice provided the first convincing evidence that IR-initiated tissue injury is complement-dependent [8,9].

The fact that *Rag-2*−/− mice lacking serum Ig are protected from IR damage and that this protection is abrogated with infusion of wild-type Ig indicated that natural antibodies play a critical role in augmented IR injury [8,9]. Further, evidence that *Cr 2*−/− mice displaying altered Ig responses were also

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resistant to IR injury despite normal circulating levels of IgG and IgM, and that injury was restored with Ig from wild-type mice, suggested the presence of a distinct subset of Abs responsible for the initiation of IR injury [10,11].

Recent work has further narrowed the pathogenic natural Ig repertoire and revealed likely initial target antigens. An antibody directed against non-muscle myosin heavy chain type II (NMHC-II) was found to reconstitute damage in IR-resistant mice [12–14] as were anti-DNA, anti-histone [15], and anti-phospholipid antibodies [16]. It appears that multiple specificities are involved in Ag-Ab reactions leading to IR damage, and with the number of downstream events in the cascade, various targets for intervention exist.

Therapeutic inhibition of the complement system has been tried successfully in multiple animal models of IR injury [2–6,17–20]. However, indiscriminate suppression of complement may increase susceptibility to infection, especially in already immunocompromised individuals. Concern over infectious risks of systemic complement inhibition led to design of complement inhibitors directed to sites of complement activation. A fusion protein consisting of complement receptor 2 and a complement inhibitor protein (Crry) effectively limited organ injury without altering the susceptibility of mice to sepsis [21].

Decay-accelerating factor (DAF) is a glycosylphosphatidylinositol (GPI)-anchored membrane protein which contains four short consensus repeat (SCR) modules and a C-terminal O-glycosylated extension [22]. DAF binds CD97 through its first SCR [23]. Through its 2nd and 3rd SCR, DAF binds to and dissociates C3 and C5 convertases assembling on host cells [22–42], thereby protecting cells from complement activation on their surfaces and preventing C5b-9 formation [25,26,43–51]. Because DAF accelerates convertase decay and interrupts the complement amplification loop, we reasoned that both local and, more prominently, remote injury should be attenuated by DAF treatment.

Our results demonstrate that administration of soluble human DAF reduces remote intestinal and lung damage in a hindlimb IR model as well as remote injury in a mesenteric IR model in normal and autoimmunity prone mice while conserving resistance to polymicrobial sepsis.

Materials and methods

Animals

C57Bl/6 mice ages 8 to 12 weeks were used in the hindlimb model of skeletal muscle IR. B6.MRL/*lpr* autoimmune mice were used in the IR model at age 5 to 6 months (after their autoimmune phenotype had sufficient time to express). Animals in this study were maintained in accordance with the guidelines of the Laboratory Animal Medicine Department/IACUC of USUHS and the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Hindlimb model of IR

C57Bl/6 mice ages 8–12 weeks or B6.MRL/*lpr* mice ages 5–6 months were exposed to 2 h of hindlimb ischemia and 3 h of reperfusion as previously described [8,13]. Anesthesia was administered with injection of a mixture of ketamine and xylazine. Two rubber bands (Latex-O-Rings; Miltex, Inc.) were applied above the greater trochanter of one hindlimb following 2 min of hindlimb elevation to decrease venous congestion using a McGivney Hemorrhoid Ligator (Miltex, Inc., York, PA). Sham mice did not undergo banding. Mice were kept anesthetized for the duration of the experiment. Five minutes prior to reperfusion, mice were injected with the indicated dose per animal DAF (rhCD55/DAF, R&D Systems, Minneapolis, MN) or 0.2 ml sterile 1× PBS intravenously by tail vein injection. At the 2-h time point, rubber bands were cut, and limb reperfusion was confirmed by return of pink color to formerly dusky ischemic limbs. Animals were allowed to reperfuse for 3 h under anesthesia prior to sacrifice. Harvested tissues (lung, intestine, liver, kidney, spleen, muscle) were fixed overnight in 10% formalin (for paraffin blocks) or 4% paraformaldehyde (for frozen sections). Frozen section tissues stored in 4% paraformaldehyde were washed in PBS and sucrose and made into blocks for future sectioning.

Determination of DAF deposition

To determine time in circulation of rhCD55/DAF, C57Bl/6 mice were subjected to hindlimb IR as described above. After 2 h of hindlimb ischemia, mice were injected with either PBS vehicle control or 2 μg DAF by tail vein injection 5 min prior to reperfusion. Tissues were harvested 5 min post-reperfusion (10 min after DAF or PBS injection) and 55 min post-reperfusion (1 h after DAF or PBS injection). Muscle, intestine, and lung were harvested and placed in 4% paraformaldehyde overnight for fixation. Organs were placed into blocks to obtain frozen sections. Sections were cut and stained with anti-DAF or anti-C3-FITC.

Mesenteric model of IR

Five-month-old B6.MRL/*lpr* and C57Bl/6 mice were exposed to laparotomy and clamping of the superior mesenteric artery (SMA) following 30 min of equilibration after opening the abdomen as previously described [15]. Clamp was released after 30 min and animals were allowed to reperfuse for 2 h prior to sacrifice by anesthetic overdose. Five minutes prior to reperfusion, mice were injected with 2 μg per animal DAF (rhCD55/DAF, R&D Systems, Minneapolis, MN) or 0.2 ml sterile 1× PBS intravenously by tail vein injection. Mice were kept anesthetized for the duration of the experiment. Harvested tissues (lung, intestine, liver, kidney, spleen, muscle) were fixed overnight in 10% formalin (for paraffin blocks) or 4% paraformaldehyde (for frozen sections). Frozen section tissues stored in 4% paraformaldehyde were washed in PBS and sucrose and made into blocks for future sectioning.

Cecal ligation and puncture (CLP) model of polymicrobial sepsis

C57Bl/6 mice ages 3–8 weeks were allowed to acclimate to the USUHS animal facility for 1–2 weeks prior to use in experiments. Mice were anesthetized with 4% isoflurane in a sealed canister using VetEquip inhalant anesthesia equipment (VetEquip, Incorporated, Pleasanton, CA). Following induction of anesthesia, mice were moved to nose cones with