RAPID COMMUNICATION

Interleukin-8 Production in Red Blood Cell Incompatibility

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Hemolytic transfusion reactions (HTR) are characterized by fever, shock, organ system failure, intravascular coagulation, and possibly death. The same findings may be associated with sepsis. Neutrophils have been implicated in the pathogenesis of HTR, although a mechanism for neutrophil activation has not been shown. In addition, the possible role that cytokines may play in HTR has not been investigated. We show that interleukin-8 (IL-8), a cytokine with chemotactic and neutrophil-activation properties, is produced in whole blood following addition of ABO-incompatible red blood cells, in a dose- and time-dependent manner related to the degree of hemolysis, and is inhibited by inactivation of complement. IL-8 production is accompanied by increased gene expression in the buffy coat. This observation has implications for the understanding of the pathogenesis of and for the treatment of HTR.

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MATERIALS AND METHODS

Blood. Heparinized whole blood (WB) was obtained from group O, and RBC were obtained from groups A, B, and O normal blood donors. ABO groups of WB and RBC were confirmed by standard hemaggultination techniques. Donors of WB lacked unexpected RBC antibodies. RBC were washed three times in normal saline, and the buffy coat removed. WB was placed in 2-mL aliquots in polypropylene tubes, to which packed RBC (hematocrit 0.82 ± 0.02, 4.15 ± 0.23 x 10^11 RBC/mL) were added in amounts given in Results. Tubes were placed on a rocker in a 37°C incubator with 5% CO2 for times indicated in Results. Tubes were reacted exclusively with rIL-8 in Western blot analysis. The assay sensitivity was greater than 50 pg/mL.

Northern Blot. Total cellular RNA was extracted using modifications of previously published methods. Brieﬂy, cells were solubilized in a solution of 25 mmol/L Tris, pH 8.0, containing 4.2 mol/L guanidine isothiocyanate, 0.5% Sarkosyl, and 0.1 mol/L β-mercaptoethanol. After homogenization, an equal volume of 100 mmol/L Tris, pH 8.0, containing 1.0% sodium dodecyl sulfate (SDS) and 10 mmol/L EDTA was added, and the RNA extracted with chloroform-phenol. The alcohol-precipitated RNA was separated by formaldehyde/1% agarose gel electrophoresis, and transblotted to nitrocellulose. The baked blots were prehybridized, and hybridized with the 32P-labeled 30-mer oligonucleotide probe 5'-GTT-GGC-GCA-GTG-TGG-TCC-ACT-CTC-ATT-CAC-3'. Blots were stringency washed and autoradiographed. Equivalent amounts of total RNA load per lane were assessed by comparing 28S and 18S rRNA.

RESULTS

Hemolysis was seen when incompatible (group A or B) RBC were added to WB, but not when compatible group O RBC were added. Hemolysis was greatest at a dose of 6 µL/mL WB (Fig 1A). Significant differences (paired t-test, P < .01) were present in the range of 3 to 25 µL/mL WB. Hemolysis was complete at 2 hours and did not further increase, although some incremental hemolysis was observed described. Polyclonal antihuman IL-8 antiserum was produced by immunization of rabbits with recombinant IL-8 (rIL-8) in multiple intradermal sites with complete Freund’s adjuvant. IL-8 antiserum reacted exclusively with rIL-8 in Western blot analysis. The assay sensitivity was greater than 50 pg/mL.

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Submitted August 27, 1990; accepted September 19, 1990.

Supported in part by National Institutes of Health Grant Nos. HL102401, HL31693, and HL35276. S.L.K. is an Established Investigator of the American Heart Association. R.M.S. is an RJR Nabisco Research Scholar.

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in all samples at 24 hours (Fig 1B). Significant differences ($P < .01$) were present at 2 to 8 hours. Equivalent hemolysis resulted from addition of either group A or group B RBC (data not shown). The addition of human rIL-8 to WB at doses up to 10 ng/mL did not cause any detectable hemolysis (data not shown).

Production of IL-8 in group O WB was observed with addition of ABO-incompatible RBC, but not with group O RBC (Fig 2A). Significant differences ($P < .01$) were seen at 3 to 50 μL/mL WB. The dose dependency of IL-8 production paralleled hemolysis, and was greatest at the same dose that maximal hemolysis occurred. IL-8 appeared in plasma in a manner progressive with time, first observed at 2 hours and increasing over a 24-hour period (Fig 2B). Significantly higher levels of IL-8 ($P < .01$) were generated in response to group A RBC as compared with group B RBC, consistent with the known higher titer of anti-A in Caucasians. IL-8 mRNA was strongly expressed when incompatible RBC were added to WB, but it was undetectable when group O RBC were added (Fig 4), indicating that gene transcription is markedly increased.

To determine if the observed response was complement dependent, separated plasma was pretreated with heating to 56°C for 1 hour before being added to the washed cellular elements of WB (reconstituted WB). Hemolysis was abolished, although isoantibodies were still present as shown by direct agglutination. IL-8 concentration in reconstituted WB was greater than in untreated WB, but failed to increase with addition of either group A or group O RBC, although LPS treatment (10 μg/mL) caused a marked response (Fig 3). Significant differences ($P < .05$) were present between untreated WB with group A RBC and reconstituted WB with either compatible, incompatible, or no RBC.

Finally, to determine whether the observed IL-8 production was associated with increased gene transcription or augmented translation of constitutive IL-8 messenger RNA (mRNA), WB was treated with 6 μL/mL of either group A or group O RBC, and buffy coat cellular RNA isolated at 2 hours for Northern blot analysis. IL-8 mRNA was strongly expressed when incompatible RBC were added to WB, but it was undetectable when group O RBC were added (Fig 4), indicating that gene transcription is markedly increased.
IL-8 IN RBC INCOMPATIBILITY

Fig 3. Effect of pretreatment of plasma with heat (56°C, 1 hour) with subsequent addition of incompatible (group A) RBC, compatible (group O) RBC, or LPS, compared with untreated WB with and without addition of incompatible RBC.

DISCUSSION

Hemolytic transfusion reactions because of incompatibility within the ABO system represent one of the most severe emergencies in transfusion medicine. Despite the significance of these reactions, the pathophysiology is poorly understood. One of the difficulties in elucidating the mechanisms underlying HTR is the lack of an in vitro model. Therefore, we developed a WB model of ABO incompatible RBC transfusion. This model has expected characteristics of hemolysis of exogenous (donor) RBC. The observed dose dependence, with maximal hemolysis at relatively small amounts of incompatible RBC, suggests that a critical intermediate, probably a complement component, is exhausted in this closed system at higher doses.

Previous concepts of the pathogenesis of HTR have focused primarily on the role of complement in causing hemolysis, production of the anaphylotoxins C3a and C5a, and the interaction with the coagulation and kinin systems. In addition, activation of neutrophils has been implicated in initiating intravascular coagulation, and in transfusion-related acute lung injury. Specific mechanisms by which neutrophils may be activated in transfusion reactions have not been established. While the complete role of cytokines in HTR remains to be determined, we have shown the production of a major cytokine, IL-8, in ABO incompatibility, which has implications for the understanding of the pathogenesis of HTR.

IL-8 is an important chemotactic cytokine. It is produced by a variety of cell types, including monocytes, alveolar macrophages, endothelial cells, and fibroblasts, in response to bacterial LPS, IL-1, and TNF-α. Its production in response to immune hemolysis suggests that an independent mechanism of activation may exist. The observed dose dependency, with IL-8 production parallelling hemolysis, and the requirement of a heat-sensitive factor in plasma, suggests that the response is mediated through a complement-dependent pathway. A marked increase in IL-8 mRNA in the buffy coat following addition of incompatible RBC, and lack of detectable constitutive IL-8 mRNA, indicates that the response occurs at the level of gene expression.

Established biologic activities of IL-8 are the recruitment and activation of neutrophils, including the release of secretory vesicles, specific granules, and azurophil granules, and induction of the respiratory burst. Neutrophils may play a role in the organ damage and initiation of intravascular coagulation seen in HTR through release of thromboplastic substances. Other possible events of neutrophil activation in HTR are oxygen metabolite production, release of proteolytic enzymes, and attachment of endothelial cells. The demonstration of IL-8 production in ABO incompatibility clearly opens a new avenue for the study of these reactions.

Fig 4. Northern blot analysis of buffy coat cell RNA for IL-8 mRNA following addition of incompatible (group A) RBC (lane 2) or compatible (group O) RBC (lane 1).
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