Hemoglobin-Specific Antibody in a Multiply Transfused Patient With Sickle Cell Disease

By Peter A. Noronha, Loyda N. Vida, C. Lucy Park, and George R. Honig

Human hemoglobins (Hbs) are known to be immunogenic, and both normal and variant forms of Hb have been shown to stimulate antibody formation in a variety of animal species. In patients who are homozygous for the sickle Hb (HbS) mutation, transfusion of normal, HbA-containing erythrocytes provides a potential stimulus for Hb alloimmunization. We tested serum samples for the presence of anti-Hb antibody by a solid-phase enzyme-linked immunosorbent assay (ELISA) using Hb-coated polystyrene microtiter plates. Hb-bound antibody was identified using an antihuman IgG antibody. Serum samples from 89 patients with sickle cell disease were initially tested for evidence of Hb antibody. The serum from three individuals exhibited antibody activity against HbA with little or no activity against HbS. Only one of these individuals was available for more study. When this patient’s antibody was tested against a variety of normal and mutant Hbs using antibody either to human IgG or to \( \kappa \) chains, the anti-Hb antibody demonstrated specificity for the region of the Hb \( \beta \) chain corresponding to the site of the amino acid substitution of HbS. The level of activity of the patient’s anti-Hb antibody showed no significant change over 1.5 years of observation. The transfusion of erythrocytes containing Hb structurally different from that of the recipient appeared to be capable of stimulating the production of Hb-specific alloimmune antibody.

From the Department of Pediatrics, The University of Illinois College of Medicine, Chicago, IL.

Submitted June 13, 1996; accepted October 22, 1996.

\( \alpha \) chain antibodies

Hemoglobin-Specific Antibody in a Multiply Transfused Patient With Sickle Cell Disease

By Peter A. Noronha, Loyda N. Vida, C. Lucy Park, and George R. Honig

Human hemoglobins (Hbs) are known to be immunogenic, and both normal and variant forms of Hb have been shown to stimulate antibody formation in a variety of animal species. In patients who are homozygous for the sickle Hb (HbS) mutation, transfusion of normal, HbA-containing erythrocytes provides a potential stimulus for Hb alloimmunization. We tested serum samples for the presence of anti-Hb antibody by a solid-phase enzyme-linked immunosorbent assay (ELISA) using Hb-coated polystyrene microtiter plates. Hb-bound antibody was identified using an antihuman IgG antibody. Serum samples from 89 patients with sickle cell disease were initially tested for evidence of Hb antibody. The serum from three individuals exhibited antibody activity against HbA with little or no activity against HbS. Only one of these individuals was available for more study. When this patient’s antibody was tested against a variety of normal and mutant Hbs using antibody either to human IgG or to \( \kappa \) chains, the anti-Hb antibody demonstrated specificity for the region of the Hb \( \beta \) chain corresponding to the site of the amino acid substitution of HbS. The level of activity of the patient’s anti-Hb antibody showed no significant change over 1.5 years of observation. The transfusion of erythrocytes containing Hb structurally different from that of the recipient appeared to be capable of stimulating the production of Hb-specific alloimmune antibody.

From the Department of Pediatrics, The University of Illinois College of Medicine, Chicago, IL.

Submitted June 13, 1996; accepted October 22, 1996.

\( \alpha \) chain antibodies

Hemoglobin-Specific Antibody in a Multiply Transfused Patient With Sickle Cell Disease

By Peter A. Noronha, Loyda N. Vida, C. Lucy Park, and George R. Honig

Human hemoglobins (Hbs) are known to be immunogenic, and both normal and variant forms of Hb have been shown to stimulate antibody formation in a variety of animal species. In patients who are homozygous for the sickle Hb (HbS) mutation, transfusion of normal, HbA-containing erythrocytes provides a potential stimulus for Hb alloimmunization. We tested serum samples for the presence of anti-Hb antibody by a solid-phase enzyme-linked immunosorbent assay (ELISA) using Hb-coated polystyrene microtiter plates. Hb-bound antibody was identified using an antihuman IgG antibody. Serum samples from 89 patients with sickle cell disease were initially tested for evidence of Hb antibody. The serum from three individuals exhibited antibody activity against HbA with little or no activity against HbS. Only one of these individuals was available for more study. When this patient’s antibody was tested against a variety of normal and mutant Hbs using antibody either to human IgG or to \( \kappa \) chains, the anti-Hb antibody demonstrated specificity for the region of the Hb \( \beta \) chain corresponding to the site of the amino acid substitution of HbS. The level of activity of the patient’s anti-Hb antibody showed no significant change over 1.5 years of observation. The transfusion of erythrocytes containing Hb structurally different from that of the recipient appeared to be capable of stimulating the production of Hb-specific alloimmune antibody.

From the Department of Pediatrics, The University of Illinois College of Medicine, Chicago, IL.

Submitted June 13, 1996; accepted October 22, 1996.

\( \alpha \) chain antibodies

Hemoglobin-Specific Antibody in a Multiply Transfused Patient With Sickle Cell Disease

By Peter A. Noronha, Loyda N. Vida, C. Lucy Park, and George R. Honig

Human hemoglobins (Hbs) are known to be immunogenic, and both normal and variant forms of Hb have been shown to stimulate antibody formation in a variety of animal species. In patients who are homozygous for the sickle Hb (HbS) mutation, transfusion of normal, HbA-containing erythrocytes provides a potential stimulus for Hb alloimmunization. We tested serum samples for the presence of anti-Hb antibody by a solid-phase enzyme-linked immunosorbent assay (ELISA) using Hb-coated polystyrene microtiter plates. Hb-bound antibody was identified using an antihuman IgG antibody. Serum samples from 89 patients with sickle cell disease were initially tested for evidence of Hb antibody. The serum from three individuals exhibited antibody activity against HbA with little or no activity against HbS. Only one of these individuals was available for more study. When this patient’s antibody was tested against a variety of normal and mutant Hbs using antibody either to human IgG or to \( \kappa \) chains, the anti-Hb antibody demonstrated specificity for the region of the Hb \( \beta \) chain corresponding to the site of the amino acid substitution of HbS. The level of activity of the patient’s anti-Hb antibody showed no significant change over 1.5 years of observation. The transfusion of erythrocytes containing Hb structurally different from that of the recipient appeared to be capable of stimulating the production of Hb-specific alloimmune antibody.

From the Department of Pediatrics, The University of Illinois College of Medicine, Chicago, IL.

Submitted June 13, 1996; accepted October 22, 1996.

\( \alpha \) chain antibodies

Hemoglobin-Specific Antibody in a Multiply Transfused Patient With Sickle Cell Disease

By Peter A. Noronha, Loyda N. Vida, C. Lucy Park, and George R. Honig

Human hemoglobins (Hbs) are known to be immunogenic, and both normal and variant forms of Hb have been shown to stimulate antibody formation in a variety of animal species. In patients who are homozygous for the sickle Hb (HbS) mutation, transfusion of normal, HbA-containing erythrocytes provides a potential stimulus for Hb alloimmunization. We tested serum samples for the presence of anti-Hb antibody by a solid-phase enzyme-linked immunosorbent assay (ELISA) using Hb-coated polystyrene microtiter plates. Hb-bound antibody was identified using an antihuman IgG antibody. Serum samples from 89 patients with sickle cell disease were initially tested for evidence of Hb antibody. The serum from three individuals exhibited antibody activity against HbA with little or no activity against HbS. Only one of these individuals was available for more study. When this patient’s antibody was tested against a variety of normal and mutant Hbs using antibody either to human IgG or to \( \kappa \) chains, the anti-Hb antibody demonstrated specificity for the region of the Hb \( \beta \) chain corresponding to the site of the amino acid substitution of HbS. The level of activity of the patient’s anti-Hb antibody showed no significant change over 1.5 years of observation. The transfusion of erythrocytes containing Hb structurally different from that of the recipient appeared to be capable of stimulating the production of Hb-specific alloimmune antibody.

From the Department of Pediatrics, The University of Illinois College of Medicine, Chicago, IL.

Submitted June 13, 1996; accepted October 22, 1996.

\( \alpha \) chain antibodies

Hemoglobin-Specific Antibody in a Multiply Transfused Patient With Sickle Cell Disease

By Peter A. Noronha, Loyda N. Vida, C. Lucy Park, and George R. Honig

Human hemoglobins (Hbs) are known to be immunogenic, and both normal and variant forms of Hb have been shown to stimulate antibody formation in a variety of animal species. In patients who are homozygous for the sickle Hb (HbS) mutation, transfusion of normal, HbA-containing erythrocytes provides a potential stimulus for Hb alloimmunization. We tested serum samples for the presence of anti-Hb antibody by a solid-phase enzyme-linked immunosorbent assay (ELISA) using Hb-coated polystyrene microtiter plates. Hb-bound antibody was identified using an antihuman IgG antibody. Serum samples from 89 patients with sickle cell disease were initially tested for evidence of Hb antibody. The serum from three individuals exhibited antibody activity against HbA with little or no activity against HbS. Only one of these individuals was available for more study. When this patient’s antibody was tested against a variety of normal and mutant Hbs using antibody either to human IgG or to \( \kappa \) chains, the anti-Hb antibody demonstrated specificity for the region of the Hb \( \beta \) chain corresponding to the site of the amino acid substitution of HbS. The level of activity of the patient’s anti-Hb antibody showed no significant change over 1.5 years of observation. The transfusion of erythrocytes containing Hb structurally different from that of the recipient appeared to be capable of stimulating the production of Hb-specific alloimmune antibody.
years he has had hypertension (blood pressure before treatment, 190/94 mm Hg). He has had at least five major surgical procedures with general anesthesia. His present medications include hydrochlorothiazide, nifedipine, and a variety of analgesics; he has received many different antibiotics on numerous occasions.

A representative blood cell count from this patient, more than 3 months after any transfused blood, showed the following: Hb, 80.0 g/L; red blood cell count, 3.40 × 10^{12}/L; mean corpuscular volume, 91 fL; reticulocyte count, 7.8%; platelet count, 210 × 10^{9}/L; and white blood cell count, 10.8 × 10^{9}/L, with 50% neutrophils, 28% lymphocytes, 16% monocytes, 4% eosinophils, and 2% basophils. His recent transfusion history is summarized in Table 1. His blood group phenotype is [B, ccDee, Ms/Ns, P_{1}, J_{k}, F_{y}^b, L_{eb}]. Despite the extensive number of previous transfusions and the considerable likelihood that he has received at least some transfused erythrocytes with blood group antigen incompatibility, he has never shown evidence of blood group alloimmunization, and his direct antiglobulin test has always remained negative.

This patient also has not shown any indication of autoimmune disease. His anti–nuclear antibody test has consistently been negative, and screening tests for circulating antibody against rheumatoid factor and smooth muscle also were negative.

**Properties of the patient’s anti-Hb antibodies.** IgG prepared from the patient’s serum demonstrated antibody activity against HbA but little or no activity against HbS in ELISAs that used goat antihuman IgG as the developing antibody (Fig 1). This specificity was also observed in assays that used antihuman κ chain antibody (Fig 2A). However, in ELISAs in which antihuman λ chain antibody was used as the developing antibody, the patient’s IgG showed binding activity to both HbA and HbS (Fig 2B).

**Antibody-binding specificity against other human Hbs.** To gain some definition of the antigen-binding epitope of the HbA-specific antibody, we substituted a number of other purified human Hbs in the ELISA. These included fetal Hb (HbF), HbA_{2}, HbG_{Philadelphia} (an α-chain variant with structurally normal β chains), HbC (β^δ Lys), Hb-Leiden (β^β or β^6 Glu deleted), and HbE (β^α Lys). The results of representative experiments are shown in Fig 3.

In the studies that used other Hb variants with structural changes at the β^α position (the site of the β^α mutation), namely HbC and Hb-Leiden, negligible antibody activity was detected with either antihuman IgG antibody or anti–κ chain antibody, with the data points being virtually indistinguishable from those obtained with HbS. With HbG and HbE, whose β^β regions are structurally normal, the antibody exhibited activity of a similar degree as against HbA (Fig 3).

Results obtained with HbA_{2} and HbF are also shown in Fig 3. With HbA_{2}, the activity of the patient’s anti-Hb antibody was approximately intermediate between that against HbA and against HbS. HbA_{2} contains α subunits that are identical to those of HbA; the sequence of amino acid residues 1 to 8 of its δ chains is also identical to those of the β chains of HbA, but at amino acid positions 9 and 12 their sequences differ. HbF also contains α subunits that are identical to those of HbA, but the amino acid sequence of its γ chains is substantially different from that of β^α; a notable difference is that the γ^α position is occupied by aspartate and β^6 by glutamate. Correspondingly, the patient’s HbA-specific antibody exhibited no apparent activity against HbF (Fig 3).

The specificity of the antibody-binding epitope of the patient’s anti-Hb IgG was also examined in an experiment that used a blocking antibody. For this purpose, mouse monoclonal antibody with antigenic specificity for β^6 to β^α of human HbA^{17} was used. When preparations of this antibody (Immu-no-rx, Augusta, GA) were added to wells containing HbA, subsequent binding of antibody from the patient’s IgG was completely inhibited. Nonimmune mouse IgG exhibited no blocking effect.

When antihuman λ chain antibody was used as the developing antibody in the ELISA, the data points from the patient’s IgG showed no significant difference from those against HbA and HbS (Fig 2B) when HbF, HbA_{2}, HbG,
HbC, Hb-Leiden, and HbE were substituted (data not shown). Each produced a virtually identical curve.

**DISCUSSION**

The antibody activity identified in this study represents, to our knowledge, the first known demonstration of human antibody formation against Hb. Earlier efforts by Reichlin to identify HbA antibody in multiply transfused HbSS patients yielded only negative results.

The patient in the present study appeared to have at least two distinct anti-Hb antibodies. One of these, an IgG with \( \kappa \) light chains, exhibited no apparent specificity against any of the various normal and mutant Hbs we examined. This antibody could have specificity for an epitope on the Hb \( \alpha \) subunits, or on a region of the \( \beta \) subunits relatively distant from the sites of the \( \beta^6 \) or \( \beta^c \) mutations, but we have no experimental evidence to address these possibilities directly. Inasmuch as there was no apparent indication of the presence of this seemingly nonspecific anti-Hb antibody in the ELSAs that employed antihuman IgG antibody (Fig 1), it would appear either that the antihuman IgG antibody we used had preferential binding to IgG with \( \kappa \) chains rather than \( \lambda \) chains, or that the patient’s anti-Hb IgG that contained \( \lambda \) chains represented only a minor component of his anti-Hb antibody.

The other anti-Hb antibody identified in this patient, in which the light chains are of the \( \kappa \) type, exhibited many of the features of an alloantibody: the patient’s major adult Hb is of the sickle cell type, and therefore does not contain the normal \( \beta^6 \) glutamate; he has been exposed repeatedly to HbA from his multiple transfusions; and this antibody displays a high order of specificity for structurally normal Hb in the region corresponding to the site of the sickle mutation.

We also consistently observed that this antibody exhibited activity against normal HbA, but of a lesser degree than that against HbA (Fig 3). The \( \beta \) chains of HbA and the \( \delta \) chains of HbA share identical amino acid sequences for amino acid positions 1 through 8, including the presence of glutamate at position 6, which is the site of the \( \beta^6 \) mutation.
At sequence positions 9, 12, and 22, the $\beta$ and $\delta$ chains contain different amino acids, with the structures of their amino termini otherwise being the same as far as amino acid position 49. From these considerations, it would appear that the antigen-binding epitope of this antibody encompasses, at a minimum, amino acid positions $\beta^6$ through $\beta^9$, and could also include position $\beta^{12}$.

Studies of antibody formation against human HbA in animals$^{4,11}$ have identified the $\beta^6$ site as being potentially immunogenic. A murine monoclonal antibody with specificity for this region has been shown to be nonreactive against HbS, HbF, HbC, and HbA$_2$, and another mouse monoclonal antibody with anti-HbC activity showed no cross-reactivity with HbA, HbS, HbA$_2$, or HbF$^6$. The $\beta$-binding properties of the $\kappa$ chain—specific antibody from our patient exhibited a degree of specificity similar but apparently nonidentical to that observed with these murine monoclonal anti-Hb antibodies.

Our patient was one of only three among 89 patients with sickle cell disease that we screened in whom we could detect the presence of Hb antibody. This apparently rare event could possibly be accounted for by the unusually large number of blood transfusions this patient received; however, many of the other adults with sickle cell disease that we studied also had a history of multiple blood transfusions. Considering the findings that suggest that this patient’s anti-Hb antibody may have arisen by an alloimmune process, it is of interest that he did not show evidence of blood group alloimmunity. This is particularly notable in light of his relatively rare Rh phenotype. Studies of patients with sickle cell disease who have been transfused have shown that the rate of blood group alloimmunization may exceed 30%.$^{20-22}$ This patient’s clinical and laboratory findings also provide no indication of his having any other form of autoimmune or immune complex disease.

In mice, the development of anti-Hb antibody has been shown to be related to the presence of specific immune response genes.$^{23}$ Whether comparable forms of genetic control might play a role in human anti-Hb antibody formation can at present only be a point of speculation.

Over the period of about 1.5 years that we studied our patient, the activity of his anti-Hb antibody remained virtually unchanged despite his having been transfused during this interval. There has been no indication that the presence of the antibody has contributed to the severity of his sickle cell disease, or to a shortening of survival of transfused erythrocytes.

ACKNOWLEDGMENT

We thank Dr Mabel Koshy for allowing us to include her patients in this study, and for her advice and help.

REFERENCES


2. Reichlin M: Quantitative immunological studies on single amino acid substitution in human hemoglobin: Demonstration of specific antibodies to multiple sites. Immunochemistry 11:21, 1974


5. Garver FA, Baker MM, Grenett HE: Immunochemical properties of abnormal hemoglobins C-Harlem ($\beta^6$Glu $\rightarrow$ Val, $\beta^{12}$ Asp $\rightarrow$ Asn), S ($\beta^6$Glu $\rightarrow$ Val), Korfhe Bu ($\beta^6$ Asp $\rightarrow$ Asn), Vancouver ($\beta^6$Asp $\rightarrow$ Tyr), and Mobile ($\beta^9$ Asp $\rightarrow$ Val). Biochem Biophys Acta 624:286, 1980


22. Tahhan HR, Holtbrook CT, Bradly LR, Brewer LD, Christie JD: Antigen-matched donor blood in the transfusion management of patients with sickle cell disease. Transfusion 34:562, 1994

Hemoglobin-Specific Antibody in a Multiply Transfused Patient With Sickle Cell Disease

Peter A. Noronha, Loyda N. Vida, C. Lucy Park and George R. Honig