The interference of antibodies to factor VIII coagulant protein (VIII:C) of 9 nonhemophilic patients with the binding to factor VIII coagulant antigen (VIII:CAg) of a reference hemophilic $^{125}$I-Fab' reagent, used in a liquid phase VIII:CAg assay, was studied. The binding competition was estimated from immunoradiometric assay (IRMA) dose–response slope of VIII:CAg present in patient plasma, interference of antibodies with the $^{125}$I-Fab' binding to VIII:CAg in normal plasma, and the displacement of antibody from the complexes with VIII:CAg by the $^{125}$I Fab'. Antibody populations from three patients were studied in detail; in the VIII:CAg assay, two of them interfered with the $^{125}$I-Fab' binding, and one did not (patient 1). The formation of stable complexes between antibodies of each patient and VIII:CAg was demonstrated by protein-A-Sepharose adsorption. The $^{125}$I-Fab' binding to VIII:CAg-anti-VIII:CAg IgG complexes indicated that patient 1 antibodies and the $^{125}$I-Fab' recognized different antigenic determinants, whereas the other two patient antibodies and $^{125}$I-Fab' recognized closely related or identical VIII:CAg determinants. These results demonstrate an apparently selective recognition of at least two distinct VIII:CAg determinants by naturally occurring antibodies, suggesting a possibility of a wider use of these antibodies in studies of the structure and function of factor VIII.

ANTIBODIES to factor VIII coagulant protein (VIII:C) occur as alloantibodies in multitransfused hemophiliacs and as autoantibodies in nonhemophilic subjects who have not previously received blood or blood products. In general, the alloantibodies present a fairly homogeneous group, inactivating VIII:C progressively and completely, whereas some autoantibodies show an unusual biphasic reaction of VIII:C inhibition and an apparently paradoxical coexistence of active VIII:C in the presence of an antibody excess.1–4

Both alloantibodies5–7 and autoantibodies8–12 have been used as reagents for immunoradiometric assay (IRMA) of factor VIII coagulant antigen (VIII:CAg), which occasionally produce divergent results, suggesting that antibody populations may differ in their ability to react with specific determinants of VIII:CAg.13–17

We report studies of the interaction of 9 nonhemophilic patients’ antibody populations with VIII:CAg, demonstrating the presence of at least two classes of autoantibodies to VIII:C, distinguishable on the basis of their ability to interfere with the binding to VIII:CAg of the $^{125}$I-Fab' fragments of antibodies from a hemophilic patient used in a liquid phase VIII:CAg assay.

MATERIALS AND METHODS

Plasma Samples

Plasma was prepared from blood of normal subjects and VIII:C-deficient patients, with or without an acquired inhibitor of VIII:C, collected into 1/9 volume of 3.8% trisodium citrate. A pool of plasma from 50 healthy subjects was used as reference normal plasma for VIII:C, VIII:CAg, and anti-VIII:C assays. All plasma samples and aliquots of the reference pool were stored at a temperature of $-25^\circ$C or lower. For some studies, plasma samples were defibrinated by incubation at 56°C for 1 hr, followed by the removal of the precipitate by centrifugation. This procedure also destroys the majority of VIII:CAg in plasma. Plasmas so treated are referred to as “heated plasmas.”

IgG Preparations

IgG was prepared by the caprylic acid precipitation method14 from the heated plasma of one multitransfused hemophilic patient with an anti-VIII:C titer of 2,000 U/ml and from the heated plasmas of patients with autoantibodies by affinity chromatography on Sepharose-4B-linked staphylococcal protein A.15

Preparation, Labeling, and Purification of Hemophilic Anti-VIII:CAg Fab'

IgG solution (4 mg/ml, 570 U/ml anti-VIII:C activity) was incubated at 37°C for 18 hr with pepsin (0.02 mg/mg IgG) in 0.1 M acetic acid buffer, pH 4.2, in the presence of 0.01 M cysteine, brought to pH 8 with 1 M NaOH, and gel-filtered on a column (2.6 x 90 cm) of Sephacryl-200.3 The Fab' peak was collected, concentrated to 1.85 mg/ml by dialysis against polyethylene glycol 4,000, and stored at $-80^\circ$C in aliquots. When required, 54 μg of Fab' solution (100 μg) were labeled with 1 μCi of $^{125}$I by the chloramine-T method,19 followed by removal of non-protein-bound radioactivity (100–150 μCi) on a 1.5 ml column of an anion exchange resin (Amberlite, IRN 78, Prolabo, France) equilibrated in 0.1 M NaCl, 0.1 M sodium phosphate buffer, pH 7.2, containing 10 g/liter bovine serum albumin (BSA). The labeled protein, eluted in about 2.5 ml, was incubated for 2 hr at 37°C with 125 U factor VIII:C in the form of a factor VIII concentrate (anti-hemophilic factor, Cutter Biological, Berkeley, CA) followed by Sepharose-4B gel filtration (1.6 x 43 cm column). The radiolabeled Fab' present in the $V_a$ fraction (~1% of the starting radioactivity) was dissociated from factor VIII by

From the Centre National de Transfusion Sanguine, Paris, France.

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Address reprint requests to Dr. J-P Allain, Centre National de Transfusion Sanguine, Zone d’Activité, 91943, Les Ulis Cedex, France.

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incubation for 1 hr at 37°C and at pH 2.4 and then isolated by gel-filtration on a 2.6 x 60 cm column of Sephacryl 200 in 0.15 M NaCl, 0.05 M Tris/HCl buffer, pH 6.8. The peak of radioactivity corresponding to the free anti-VIII:C \(^{125}\)I-Fab' (0.2%-0.5% of the starting radioactivity) was collected and stored at 4°C in the presence of 0.02% NaN_3.

**Measurements of VIII:C and Anti-VIII:C**

Factor VIII:C was measured by a one-stage assay.\(^{21}\) Anti-VIII:C activity was estimated as previously described.\(^{22}\) One anti-VIII:C unit, as defined here, is the amount of antibody that inhibits 75% of VIII:C contained in a 1:1 mixture of the antibody-containing solution and normal plasma during a 2-hr incubation at 37°C. One anti-VIII:C unit corresponds to approximately 2 Bethesda units. All anti-VIII:C-containing samples were also tested by inhibition of the coagulation of normal plasma incorporated in 1% agarose gel. The anti-VIII:C unit corresponds to approximately 2 Bethesda units. All anti-VIII:C-containing samples were also tested by inhibition of the coagulation of normal plasma incorporated in 1% agarose gel following the diffusion of the antibody in the gel.\(^{23}\) In order to increase the sensitivity of the assay, the normal plasma was sometimes diluted up to fourfold with hemophilic plasma.

**Liquid Phase IRMA of VIII:CAg**

Patient and standard plasma were diluted in a 0.15 M NaCl, 0.01 M imidazole buffer, pH 7.4, containing 10 mg/ml normal human IgG (CNTS-Paris). In some instances, heated plasma from a severe hemophilia A patient was used as diluent in order to assure comparability of dilutions in terms of protein concentration and composition. Duplicate 100-μl samples of each dilution were mixed with 100 μl (≈2,000 cpm) labeled anti-VIII:CAg Fab and incubated for 18 hr at room temperature (20–22°C). Each sample was then mixed with an equal volume of 76% saturated ammonium sulfate solution, left for 30 min at room temperature, and centrifuged at 3,000 g for 15 min at room temperature. Precipitates were washed twice with 100 μl of 38% saturated ammonium sulfate solution and counted for 5-10 min in a gamma counter. When plotted on log-log paper, normal reference plasma gave a linear dose-response curve in the 1/16-1/128 dilution range. The linearity of the curve was extended by logit transformation of the percentage of precipitated radioactivity.\(^{24}\) The slopes of the IRMA dose-response curves (logit percent binding versus log plasma dilution) were determined by linear regression analysis.\(^{14}\)

**Studies of the Interference of Anti-VIII:C Antibodies With the Binding of \(^{125}\)I-Fab' to VIII:CAg**

Competition between patient IgG and \(^{125}\)I-Fab' was investigated in several ways. (1) Anti-VIII:C containing IgG fractions was isolated from several patients' heated plasmas and adjusted to 9 U/ml anti-VIII:C activity and 3 mg/ml IgG, using a solution of normal human IgG. One volume of each anti-VIII:C solution was mixed with 9 volumes of normal plasma, incubated for 2 hr at 37°C, after which an IRMA dose–response analysis was performed.

(2) Heated patient plasmas were serially diluted with heated hemophilic plasma to give antibody concentrations ranging between 0.015 and 15 anti-VIII:C U/ml. Each dilution was then mixed with an equal volume of reference plasma diluted 1:10 with heated hemophilic plasma. After 1-hr incubation at 37°C, labeled Fab' was added to each tube, and the samples were treated as described for liquid phase IRMA. As a control, the competition between \(^{125}\)I-Fab' and allo-IgG from which \(^{125}\)I-Fab' was purified was tested.

To examine the presence of VIII:CAg-anti-VIII:CAg immune complexes in this system, the samples with the highest (15 U/ml) and lowest (0.015 U/ml) dilutions of antibody were prepared in quadruplicate. Two of the samples were treated as indicated above. Two were passed through a 0.6×3.6 cm Cl-Sepharose-4B-linked staphylococcal protein A column, equilibrated with 0.15 M NaCl, 0.1 M sodium phosphate buffer, pH 7.4, before the ammonium sulfate precipitation step. Elution was carried out in the same buffer. Total, as well as ammonium sulfate precipitable, nonadherent radioactivity was measured. Protein-A-adsorbed radioactivity was estimated from the difference to total sample radioactivity before and after the passage through the column and corrected for the adsorption of \(^{125}\)I-Fab' from a control mixture of \(^{125}\)I-Fab', normal plasma, and heated hemophilic plasma without anti-VIII:C.

| Table 1. VIII:C, Anti-VIII:C, VIII:CAg, and Slopes of the IRMA of VIII:CAg in Plasmas of Nonhemophilic Patients With Autoantibodies to VIII:C |
| --- | --- | --- | --- |
| Plasma Samples | VIII:C (U/ml) | Anti-VIII:C (U/ml) | VIII:CAg (U/ml) | IRMA Slopes |
| Patients | | | | |
| 1 | <0.01 | 30 | 2.60 | 2.19 |
| 2 | 0.06 | 19 | 0.53 | 2.04 |
| 3 | <0.01 | 128 | 0.13* | 1.71† |
| 4 | 0.02 | 11 | 0.58 | 2.00 |
| 5 | <0.01 | 1,200 | 0.04* | 1.65† |
| 6 | 0.01 | 8 | 0.06* | 1.45† |
| 7 | 0.24 | 0.6 | 2.45 | 2.32 |
| 8 | 0.01 | 27 | 0.47 | 2.00 |
| 9 | 0.01 | 10 | 1.36 | 2.38 |
| Controls | | | | |
| Reference normal pool (n = 21) | 1.05 | 0.99 | 2.26 ± 0.34 |
| Normal subjects (n = 21) | (0.76–1.48) | (0.58–1.66) | | |
| Hemophiliac and von Willebrand patients | 0.04 | 0.078 | 2.27 ± 0.48 |
| (n = 8) | (0.014–0.15) | (0.014–0.24) | |

*Approximate values (see text).†p < 0.05 in comparison to means of the controls.

IRMA slopes for controls: mean ± 2 SD; VIII:C and VIII:CAg for controls: mean (range); n for the reference: repeated testing of the same normal plasma pool.
Plasmas of patients with different VIII:Cag dose–response slopes were diluted with heated hemophilic plasma to comparable VIII:Cag concentration (0.01 U/ml), as estimated by the standard IRMA. One volume of plasma dilution was then mixed with 10 volumes of progressively diluted 125I-Fab' solution to obtain in the mixtures' final 125I-Fab' concentrations ranging between 0.09 and 1.8 times that used in the standard VIII:Cag assay. After 18 hr of incubation, the VIII:Cag-bound radioactivity was measured and compared to equally treated normal plasma. The displacement of alloantibodies by 125I-Fab' was also tested.

RESULTS

The results of VIII:C, anti-VIII:C, and VIII:Cag measurements in the plasmas of 9 nonhemophilic patients are shown in Table 1 and Fig. 1. The dose–response curves of VIII:Cag for all patients except patients 3, 5, and 6 were parallel to the reference plasma curve. The slopes of the dose–response curves for patients 3, 5, and 6 were significantly different from the mean dose–response curve for normal subjects, as well as for patients with hemophilia A and von Willebrand's disease with low VIII:Cag but without an anti-VIII:C (Table I). Consequently, the VIII:Cag values given in Table 1 for these patients were approximated from the intercepts of the dose–responses with the plasma dilution scale at the 5% 125I-Fab' binding level.

The relatively shallow IRMA dose–responses obtained with VIII:Cag of patients 3, 5, and 6 suggested an interference of their anti-VIII:C with the 125I hemophilic Fab' binding, which was not apparent in the remaining 6 patients. This was further studied (Fig. 2). Incubation of IgG antibody from patient 1 with normal plasma at a 1:1 U ratio of anti-VIII:C to VIII:C had no apparent effect on the binding of 125I-Fab' to VIII:Cag. In contrast, IgG antibodies from patient 3 clearly decreased the binding of the 125I-Fab', but to a lesser extent than the alloantibodies. However, the two latter antibodies caused similar alterations of the IRMA dose–response slope (from 2.2 to 1.47 and 1.58, respectively).

Figure 3 shows the effects of changes in the antibody concentration on the binding of 125I-Fab' in 1:1 mixtures of 1:10 diluted normal plasma and heated patient plasma, in which anti-VIII:C concentrations were adjusted to between 0.015 and 15 U/ml by dilution with heated hemophilic plasma. Antibody from patients 3 and 5 caused a progressive decrease in 125I-Fab' binding. A comparison with the dose–response curve showed that this corresponds to 70% and 88% decreases in VIII:Cag detection, respectively. At comparable anti-VIII:C concentrations, the alloantibody caused 98% inhibition of the VIII:Cag detection by its own labeled Fab'. In contrast, antibody from patient 1 actually caused a progressive increase in the binding of 125I-Fab'. This effect was partly due to residual VIII:Cag in the heated plasma and could be
substantially decreased by adsorption of the heated plasma with Sepharose-linked allo-anti-VIII:C (curve 1a) (heating only lowered the VIII:CAg in plasma of patient 1 from 2.6 to 0.25 U/ml, and the subsequent immunoadsorption reduced it further to 0.035 U/ml). However, both nonadsorbed and immunoadsorbed plasmas from patient 1 at 15 anti-VIII:C U/ml caused an increase in 125I-Fab' binding to VIII:CAg in 1:10 diluted normal plasma, corresponding to 0.05 and 0.07 U of VIII:CAg, respectively, in excess of the expected values. Immunoadsorption of heated plasmas of patients 3 and 5 was without an effect on the antibody interference with 125I-Fab' binding (not shown).

The formation of complexes between patient anti-VIII:C IgG and VIII:CAg was also studied by protein A adsorption. Two dilutions of plasma from patients 1, 3, and 5, containing 15 or 0.015 anti-VIII:C U/ml, were incubated with 1:10 diluted normal plasma for 1 hr at 37°C. Following incubation with 125I-Fab' solution, the samples were passed through Sepharose-linked protein A columns before the ammonium sulfate precipitation step (Table 2). When a mixture of 125I-Fab' and heated hemophilic plasma devoid of antibody was passed through the column, about 7% of the total radioactivity was retained. Incubation of hemophilic and normal plasma prior to addition of 125I-Fab' resulted in only 2% additional retention. This value is shown in Table 2, column III, as protein A-adsorbed radioactivity for the control sample. When antibody plasmas were substituted for hemophilic plasma, substantially more 125I-Fab' radioactivity now bound to the protein-A-Sepharose column (Table 2, column III). Anti-VIII:C from all three patients was completely adsorbed by protein-A-Sepharose (not shown). Thus, adsorbable radioactivity (Table 2, column III) represents 125I-Fab' binding to VIII:C-

<table>
<thead>
<tr>
<th>Table 2. Binding of Immune Complexes Formed Between Autoantibodies and VIII:CAg in Mixtures of Patients' and Normal Plasma to Protein-A-Sepharose</th>
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<tr>
<td><strong>Plasma Added to Normal Plasma</strong></td>
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<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Hemophilic control</td>
</tr>
<tr>
<td>Patient 1 (1a)</td>
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<tr>
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<td>Patient 3</td>
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The mixtures of normal and patients' plasma dilutions were prepared as described in the legend of Fig. 3. After the incubation with 125I-Fab', each sample was passed through a 1-mL column of protein-A-Sepharose. All values are percentages of total 125I-Fab' radioactivity of the sample. Values obtained with patient 1 plasma after adsorption with allo-anti-VIII:C are in parentheses.

Protein-A-adsorbed radioactivity (III): values corrected for nonspecific 125I-Fab' retention (7% of total radioactivity) in the absence of VIII:CAg. The values of nonadsorbed precipitable radioactivity (IV) when converted to relative VIII:CAg values, taking the amount applied on the column as 100%, give: 62% for control, <1% for higher concentration of each antibody, and between 20% and 30% for the lower concentration of antibodies.
anti-VIII:C complexes, while nonadsorbable, but precipitable, radioactivity (Table 2, column IV) represents $^{125}$I-Fab' bound to noncomplexed VIII:C. At a low antibody concentration (0.015 U/ml), patients 1, 3, and 5 show similar total $^{125}$I binding, roughly half bound to VIII:Cag–anti-VIII:Cag complexes (column III) and half bound to free VIII:Cag (column IV). At higher antibody concentration (15 U/ml), however, patients 3 and 5 show a distinctly different behavior than patient 1: whereas patient 1 shows an increase of total $^{125}$I-Fab' binding (column V), patients 3 and 5 show a substantial decrease. Since no free VIII:Cag is present in any of the samples at high antibody concentrations (column IV), these results further support the idea that antibodies 3 and 5 interfere with the binding of the hemophilic anti-VIII:Cag Fab', whereas antibody 1 does not.

When $^{125}$I-Fab' binding was studied in normal plasma and patients' plasma as a function of the concentration of $^{125}$I-Fab' (Fig. 4), a relatively greater increase in $^{125}$I-Fab' binding was seen in patient 3 plasma than in control and plasma from patient 1. This suggests that patient 3 antibodies could be displaced from VIII:Cag–anti-VIII:Cag complexes by increasing concentrations of Fab', but patient 1 antibodies could not. $^{125}$I-Fab' also caused some displacement of the alloantibody complexed with VIII:Cag in normal plasma but, although patient 3 and alloantibody were tested at equivalent anti-VIII:C unit concentrations, the displacement of the latter required higher $^{125}$I-Fab' concentrations.

**DISCUSSION**

Immunoradiometric assay of VIII:Cag, with its high sensitivity for the detection of both active and inactive protein, has already proved useful in prenatal diagnosis of hemophilia,24,25 in studies of genetic variants of this disease,5,12 and in the laboratory evaluation of different therapeutic preparations of factor VIII.17,26-28

The work published to date seems to support claims that both allo- and auto-anti-VIII:C antibodies are suitable reagents for the IRMA of VIII:Cag.13,29 In the absence of systematic investigation, it is not yet possible to decide whether some discrepancies in the results obtained by different laboratories could be related to the use of antibodies belonging to one or the other group. It should, however, be noted that functionally similar antibodies are likely to be selected from either group by the similar purification procedures that, in most published reports, have been based on a strong affinity binding of the antibodies to the whole, presumably native, high molecular weight factor VIII complex. Another level of selection is introduced by the practical requirement for a sufficiently high titer if an antibody population is to be a useful reagent for IRMA. Therefore, data on the VIII:Cag binding properties of lower titer antibodies, which are strongly represented in the autoantibody group,4 are still missing. Nevertheless, it might be of interest to note that at least one laboratory has reported findings that suggest some specific properties of the autoantibodies used as reagent for the IRMA of VIII:Cag not shared by most allo- and autoantibodies employed by others.11,17

The presence of variable amounts of IRMA-detectable VIII:Cag in the plasmas of a considerable proportion of patients with autoantibodies to VIII:C is an intriguing finding.7,14,15,30 Several mechanisms for this observation seem plausible. (1) The measurable VIII:Cag may represent a fraction that does not readily interact with the patient's antibodies, but freely interacts with the labeled antibodies. (2) The measurable VIII:Cag may already be bound in immune complexes with the patient's antibodies, but the labeled antibody may differ in its specificity and interact with the complexed VIII:Cag at another site. (3) Complexed VIII:Cag might be detectable because the labeled antibody is capable of displacing patient antibodies from the complex.

In order to investigate these possibilities, we measured the binding of $^{125}$I-Fab' fragments of allo-anti-VIII:C antibodies from a multitransfused hemophilic patient to the VIII:Cag present in the plasmas of 9 patients with anti-VIII:C autoantibodies and compared it with the $^{125}$I-Fab' binding to VIII:Cag in normal plasma and in mixtures of normal and patient plasma. The adsorption of immune complexes to protein-A-Sepharose was employed for a direct demonstration of the binding of $^{125}$I-Fab' to preformed VIII:Cag–anti-VIII:Cag complexes.

The IRMA dose–response slopes of VIII:Cag measurements were indistinguishable from normal in six patients and significantly less steep in the remaining three patients (Fig. 1), suggesting, in the latter group, a reduced affinity of $^{125}$I-Fab' binding. These three patients had apparently low residual VIII:Cag levels, but this in itself could not have been the reason for the shallower slopes, since in our assay, a group of hemophilic and von Willebrand's disease patients with similarly low VIII:Cag levels had normal dose–response slopes. Also, lowering of VIII:Cag concentration in normal plasma by a tenfold dilution with heated hemophilic plasma did not alter the IRMA slope. Neither could an alteration of VIII:Cag during sample storage explain the abnormal slopes, since all samples used for comparisons were equally treated. That the patients' antibodies were responsible for the apparent
low affinity of $^{125}$I-Fab' for VIII:CAg was strongly suggested by the decrease in $^{125}$I-Fab' binding to 
VIII:CAg in normal plasma upon incubation with the 
IgG fraction of plasma from patient 3 (Fig. 2). The 
change in the IRMA dose–response slope in the 
presence of autoantibodies from this patient was in fact 
comparable to the change caused by alloantibodies 
from which $^{125}$I-Fab' reagent was prepared, suggesting 
a similarity in the interaction of the two antibody 
populations with VIII:CAg. In contrast, antibodies 
that had no apparent influence on the $^{125}$I-Fab' binding 
to the VIII:CAg in patient’s plasma (patient 1) had no 
detectable influence on $^{125}$I-Fab' binding to the 
VIII:CAg in normal plasma.

The measurement of $^{125}$I-Fab' binding to VIII:CAg 
in the presence of increasing concentrations of differ-
et antibodies (Fig. 3) further amplifies these obser-
vations. Antibodies 3 and 5 showed dose-dependent 
hituation of the binding, which was, however, less 
pronounced than the inhibition caused by alloantibod-
ies from which the labeled reagent was prepared. In a 
recently published study a degree of the binding com-
petition with two different labeled Fab’ probes has 
been employed for classifying anti-VIII:C antibodies 
in terms of the recognition of the same or different 
VIII:CAg epitopes. 
Assuming similar binding affini-
ty and similar concentrations of the competing anti-
bodies, the present results would argue against the 
identity of epitopes recognized by autoantibodies of 
patients 3 and 5 and those recognized by the alloanti-
body. However, due to a known polyclonal nature of 
most anti-VIII:C autoantibodies, the antibodies that 
compete with the labeled probe may only represent a 
variable proportion of the total population of anti-
VIII:C antibodies of these patients. Since neither 
proportions nor affinities of competing antibodies have 
been established, the question of the identity of epi-
topes recognized by antibodies 3 and 5 and alloanti-
body must remain open at present.

The results of VIII:CAg retention in protein A 
columns (Table 2) suggest that all three autoantibod-
ies studied, at least when bound to protein A, formed 
similarly stable complexes with the factor VIII coagu-
ulant protein. A significant retention of VIII:CAg on 
protein A columns at a noncompeting concentration of 
patients’ 3 and 5 antibodies (0.015 U, Table 2) could 
be explained by the presence of antibodies that are 
capable of binding VIII:CAg without an interference 
with $^{125}$I-Fab' binding. An alternative possibility of a 
more effective complexing of VIII:CAg with antibod-
ies bound to protein A than with antibodies in the 
liquid phase seems less likely, since the sum of the 
$^{125}$I-Fab' bound to retained and nonretained fractions 
of VIII:CAg, at this antibody concentration, did not 
differ from control.

The demonstration of stable complex formation 
between factor VIII coagulant protein and autoanti-
bodies of patient 1 without any detectable interference 
with allo-$^{125}$I-Fab' binding suggests that these antibod-
ies and the $^{125}$I-Fab' reagent recognized different anti-
genic determinants. Moreover, antibody 1 caused a 
regular increase in $^{125}$I-Fab' binding demonstrable both 
in the liquid phase and in the complexes retained in 
protein A columns. Since this binding could not be 
fully accounted for by either residual patient 
VIII:CAg or VIII:CAg measured in normal plasma in
the absence of antibodies, it would seem that patient 1 antibodies at higher concentration also had some positive cooperative effect on $^{125I}$-Fab' binding.

Attempts to displace antibodies from complexes with VIII:CaG by increasing the $^{125I}$-Fab'/antibody ratio (Fig. 4) apparently succeeded in plasma of patient 3 and in normal plasma mixed with alloantibodies but not with plasma of patient 1.

Assuming that the IRMA dose–response slopes are a sufficiently sensitive indicator of the binding competition between the antibodies and $^{125I}$-Fab' for VIII:CaG (Fig. 1, Table 1), the antibodies of patients 7 and 9 appear to be functionally similar to those of patient 1, and antibodies of patient 6 to those of patients 3 and 5. The IRMA dose–response slopes of patients 2, 4, and 8 were at the lower limit of the corresponding control values, showing no clear predominance of a functionally distinct type of antibody in these patients.

This work has demonstrated that autoantibodies to VIII:C may represent a variety of antigenic specificities. The possible presence of at least two distinctly different types of antigenic determinant recognized by specific, naturally occurring antibodies is directly relevant to the understanding of the anti-VIII:C properties of different antibodies, as well as to a practical use of different antibodies, either as reagents in routine IRMA of VIII:CaG or as specific probes in studies of the relationship between the structure of factor VIII and the function of its coagulant part.

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Heterogeneity of autoantibodies to factor VIII: differences in specificity for apparently distinct antigenic determinants of factor VIII coagulant protein

MP Croissant, M Zuzel and JP Allain