Acquired Factor VIII Antibodies: Further Immunologic and Electrophoretic Studies

Abstract. *Seven new cases of acquired inhibitors of Factor VIII have been typed immunologically as $\gamma_2\kappa_2$ antibodies. Electrophoretic mobility on starch block of a combined group of 13 such antibodies is considerably more rapid than that of the bulk of immunoglobulin G.*

Acquired inhibitors of Factor VIII (antihemophilic globulin) have been shown to be antibodies (*1–3*). They are most commonly seen in multiply transfused patients with hereditary Factor VIII deficiency (hemophilia A), but are also observed occasionally in postpartum women, in patients with disease states associated with altered immunologic reactivity, and in some older individuals in prior good health (*4*). The presence of such inhibitors usually results in a severe hemorrhagic diathesis, unresponsive to therapy with Factor VIII concentrates (*5*). Five cases have been studied to date, in all of which the antibody was shown to be an IgG_κ immunoglobulin (*2*, *6*, *7*), although in one of these reports (*6*) some anti-Factor VIII activity was also found in an IgM fraction (Ig = immunoglobulin). We have studied seven additional cases, all of which appear to be kappa immunoglobulins. Moreover, examination of a total of 13 inhibitors by starch-block electrophoresis shows them to have a mobility much more rapid than the bulk of the gamma globulin, the majority of them migrating in the beta region.

Plasma or serum samples from patients were subjected to starch-block electrophoresis at *p*H 8.6. The block was cut into 1-cm-wide segments and the protein was eluted. Activity against Factor VIII, of each segment, was determined from the loss of Factor VIII after incubation of eluates with standard normal plasma. Inhibitors were then typed by incubating rabbit antiserums to human immunoglobulins with peak inhibitor tubes and observing neutralization of activity against Factor VIII. We have described these methods in detail elsewhere (*2*). Since the eluates themselves, probably due to the presence of a small amount of starch, were slightly clot-promoting in the Factor VIII assay, controls were run with starch-block eluates from segments not containing protein. Rabbit antiserums to human IgG, IgA, and IgM were obtained commercially. Anti-kappa and anti-lambda antiserums were obtained from several sources, and were also prepared in our own laboratory. Because of a clot-promoting effect of some rabbit antiserums, all antiserums were adsorbed with one-tenth by volume of aluminum hydroxide suspension (*8*) and incubated at 60°C for 30 minutes. This procedure did not alter antibody potency, but eliminated nonspecific effects in the Factor VIII assay. Precipitating potency of all antiserums was checked prior to use by test tube precipitation and agar-gel double diffusion against appropriate immunoglobulin antigens. It was found that the potency of anti-light-chain antiserums varied widely, both with respect to precipitin reactions and in terms of inhibitor-neutralizing capacities. Accordingly, inhibitors were considered to be exclusively kappa only when complete neutralization could be produced by anti-kappa antiserum, and no neutralization whatsoever could be demonstrated with anti-lambda antiserum. In most cases more than one set of antiserums was used for each study (*9*).

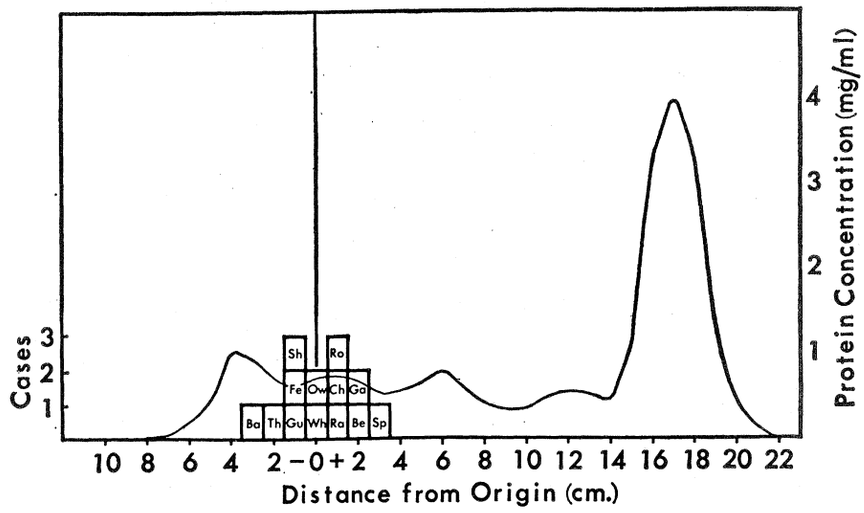

Fig. 1. Mobility of Factor VIII inhibitor peaks on starch-block electrophoresis.

Table 1. Inhibitor concentration and immunologic type of anti-Factor VIII inhibitors. Qns, quantity not sufficient for testing.

Patient	Clinical condition	Inhibitor concentration (unit/ml)	Heavy chain	Light chain
Ba	Hemophilia A	180	Gamma	Kappa
Ra	Hemophilia A	300	Gamma	Kappa
Gu	Hemophilia A	50	Qns	Kappa
Ch	Hemophilia A	65	Qns	> 98% kappa
Fe	Hemophilia A	75	Gamma	Kappa
Sh	Hemophilia A	333	Gamma	Kappa
Ow	Postpartum	12	Gamma	Kappa
Wh	Postpartum	2	Qns	Qns
Sp	Spontaneous	11	Gamma	Kappa
Be	Spontaneous	27	Gamma	Kappa
Th	Spontaneous	3	Qns	Qns
Ro	Spontaneous	115	Gamma	> 98% kappa
Ga	Ulcerative colitis	320	Gamma	Kappa

Results of these studies are summarized in Table 1. Cases Ba, Ow, and Ga were previously reported by us (*2*); case Sh was reported by Poulik and Lusher (*6*), who generously made serum samples available. In several instances immunological typing was not done or was incomplete, owing to the small amount of plasma available (Gu, Ch) or to the low level of anti-Factor VIII activity (Wh, Th). In eight cases anti-Factor VIII antibodies were clearly shown to be IgG_κ molecules. This group includes serum Sh in which we were unable to find evidence of IgM molecules with activity against Factor VIII either after starch-block electrophoresis or Sephadex G-200 gel filtration. Two additional cases cannot be said with equal certainty to be exclusively kappa (Ch, Ro): in both cases complete neutralization of activity against Factor VIII was achieved with anti-kappa antiserums, but 1 to 2 percent neutraliza-

tion of inhibitor apparently occurred with anti-lambda antiserum. Although this is a very small change in antibody titer, it represents a somewhat greater change in residual Factor VIII concentration than can be accounted for by the error of our method. Case Gu was exclusively a kappa immunoglobulin, but insufficient material was available for heavy-chain typing.

Mobility of the inhibitor peaks on starch-block electrophoresis is illustrated in Fig. 1. A typical protein pattern is shown for comparison. Only one inhibitor (Ba) migrates with the bulk of the gamma globulin. The remainder have more rapid mobilities. There is no relationship between the clinical condition in which anti-Factor VIII antibodies arose, or the serum concentration of these antibodies, and their electrophoretic mobility.

On the strength of the additional cases reported here, it seems probable that most acquired inhibitors of Factor VIII are so-called "monotypic" antibodies. Further support for this conclusion is the recent finding of a single heavy-chain subtype (γ_4) in an IgG_K anti-Factor VIII antibody (*7*). The nature of the stimulus to production of such homogeneous antibody is unknown. It is interesting to speculate on the unusually rapid mobility exhibited by this group of anti-Factor VIII antibodies. Sela and Mozes have demonstrated an inverse relationship between net antibody charge and net electrical charge of the provoking antigen (*10*). One would expect, therefore, a relatively positive electrical charge for the Factor VIII molecule at *p*H 8.6, a prediction not in keeping with its known rapid mobility on electrophoresis at this *p*H (*11*). A second possibility is suggested by the observation that human IgG4 has a much more rapid anodal mobility than the other IgG subclasses (*12*). On the basis of the mobilities exhibited by the inhibitors we have studied, it seems to us possible that many acquired anti-Factor VIII antibodies may be exclusively $IgG4_K$ immunoglobulins.

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References and Notes

1. E. Bidwell, K. W. E. Denson, G. W. R. Dike, R. Augustin, G. M. Lloyd, *Nature* **210**, 746 (1966).
2. S. Shapiro, *J. Clin. Invest.* **46**, 147 (1967).
3. H. Strauss and E. Merler, *Blood* **30**, 137 (1967).
4. A. Margolius, Jr., D. P. Jackson, O. D. Ratnoff, *Medicine* **40**, 145 (1961).
5. E. Bidwell, in *Treatment of Haemophilia and Other Coagulation Disorders*, R. Biggs and R. G. Macfarlane, Eds. (Davis, Philadelphia, 1966), pp. 93–106.
6. M. D. Poulik and J. Lusher, *Federation Proc.* **26**, 312 (1967).
7. B. R. Andersen and W. D. Terry, *Nature* **217**, 174 (1968).
8. We thank Drs. C. A. Alper, F. Hymes, H. G. Kunkel, E. Merler, R. L. Nachman, and W. Yount for their generous gifts of anti-light-chain antiserums; we also thank Drs. H. Bowman, R. L. Nachman, J. Niemetz, M. D. Poulik, and W. Scharfman for making available plasma and serum samples from their patients.
9. Cutter Laboratories, Berkeley, California.
10. M. Sela and E. Mozes, *Proc. U.S. Nat. Acad. Sci.* **55**, 445 (1966).
11. E. Bidwell, G. W. R. Dike, K. W. E. Denson, *Brit. J. Haematol.* **12**, 583 (1966).
12. H. M. Grey and H. G. Kunkel, *J. Exp. Med.* **120**, 253 (1964).
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Microstigmus comes: Sociality in a Sphecid Wasp

Abstract. *Pendent nests of the wasp* Microstigmus comes *from Costa Rica contained up to 18 adults each. Ovarian dissection indicates that there is reproductive dominance (division of labor) among females from the same nest, without apparent external morphological differences. Evidence for parental care and cooperation in provisioning and defense also identify this as the first social sphecid wasp.*

Sociality in the Hymenoptera has been achieved independently at least ten times (*1*), mostly in the Apoidea, which also exhibit many intermediate stages of social evolution. Full evolution of social behavior in wasps has been demonstrated only in the family Vespidae (*2*), and, among the Sphecidae, only a few presocial species have been reported (*3*). A census of 22 active nests from Costa Rica now gives evidence of the first fully social wasp in the family Sphecidae (*Microstigmus comes* Krombein) (*4*).

Each known species of the Neotropical genus *Microstigmus* constructs a baglike nest (Fig. 1) suspended from the underside of leaves of broad-leaved primary forest plants (*5*, *6*). Cells, each mass provisioned with Collembola, are pocketlike cavities in the lower half of the nest, and adults (Fig. 2) reside in the hollow upper portion below the entrance. Among the Sphecidae, only *Microstigmus* has a pendent nest, possibly a significant social preadaptation.

Of the 88 *Crysophila guagara* Allen palms in the study plot, 38 were occupied by a total of 74 nests: 16 had one nest; 14 had two nests; seven had three nests; one had four nests; and one had five nests. In the nests (all of which were collected at night when all adults were presumed within) were 56 females and 19 males; half the nests contained two or more females. No significant morphological differences exist between females from the same nest, nor is there dimorphism in either wing length or head width; behavioral or physiological caste differences may, however, be present without morphological correlates, as in some bees (*7*).

Table 1. Ovarian condition ranked in order of decreasing size of largest oocyte of females from eight nests of *Microstigmus comes* with three or more female adults present. There are three ovarioles per ovary, each with no more than one visible oocyte; the mature oocyte occupies nearly two-thirds of the abdomen. In four nests one female has two oocytes that are more developed than those of any of her nest mates. Parentheses indicate number of visible oocytes. NR, not recorded; U, no visible oocyte development; D, destroyed accidentally.

Cells per nest	Oocyte lengths (in mm)							
	Longest two for most mature females	Longest from other females						
4	0.75(4) .23	0.30(2)	0.18(1)					
7	.18(2) NR	.15(2)	.13(1)	0.13(2)				
4	0.38(3) .30	.15(2)	.10(1)	.08(2)				
1	.33(2) NR	.23(3)	.20(1)	.18(1)	0.15(1)			
3	0.27(1)	.20(1)	.10(2)	.10(1)	U			
10	.43(4) .18	.15(2)	.15(2)	.13(1)	0.08(1)			
9	.68(3) .25	.18(2)	.13(2)	.10(1)	U	D		
13	1.00(3) 0.55	.20(3)	.13(2)	.13(1)	0.10(2)	0.10(2)	0.08(2)	3U