Genetic Factors and Polypeptide Chain Subclasses of Human Immunoglobulin G Detected in Chimpanzee Serums

Abstract. *The Gm and Inv genetic factors, characteristic antigens of human immunoglobulin G, were detected in chimpanzee serums. All animals tested were Gm(a+, x−, b^1−, b^2−, b^3+, b^4+). Polymorphism was demonstrated for factors Gm(c), Inv(l), and Inv(b). Three of the subclasses of heavy polypeptide chains and both types of light polypeptide chains that are present in human immunoglobulin G were identified in chimpanzee serums.*

Human IgG (*1*) (7*S* γ_2-globulin) molecules consist of heavy polypeptide chains (γ-chains) and light polypeptide chains. Four antigenically different forms of γ-chains, designated γ_{2a}-, γ_{2b}-, γ_{2c}-, and γ_{2d}-chains (*2*) or Ne, We, Vi, and Ge groups (*3*), and two antigenically distinguishable forms of light chains (κ-chains and λ-chains) have been identified (*4*). Combinations of these four subclasses of γ-chains with the two types of light chains result in eight antigenically different forms of IgG molecules. This antigenic heterogeneity is detected in the serums of normal individuals with the eight molecular forms being present simultaneously in all serums.

A second type of IgG heterogeneity involves genetic polymorphism. Molecules of human IgG contain two groups of genetically determined antigenic factors, designated Gm and Inv factors (*5*). The Gm factors are associated with γ-chains (*6*, *7*) while Inv factors are detected on light polypeptide chains (*7*).

Relationships have been demonstrated between Gm and Inv factors and the antigenic subclasses of human IgG heavy and light polypeptide chains. Gm(a), (x), (b^2), and (f) are detectable only in molecules of the γ_{2b}-subclass (We group) and Gm(b^1), (b^3), (b^4), and (c) only in the γ_{2c}-subclass (Vi group) (*8*, *9*). Inv(l) (*10*) and Inv(b) are found only in molecules with κ-chains (*9*). Since some Gm factors have been detected in the chimpanzee (*11*), a more extensive study of this primate for human Gm and Inv factors, human γ-chain subclasses, and human light-chain types was undertaken. Chimpanzee serums were tested for seven Gm factors [(a), (x), (b^2), (b^1), (b^3), (b^4), (c)] and two Inv factors [(l), (b)]. In addition, selected serums were studied with antiserums detecting three subclasses of human γ-chains (γ_{2a}, γ_{2b}, and γ_{2c}) and two types of light chains (κ and λ).

Testing for Gm and Inv was done by the hemagglutination inhibition reaction (Table 1) (*12*). A total of 117 chimpanzee serums were studied (*13*). Serum samples from 64 chimpanzees were absorbed with human Type O, Rh-positive erythrocytes before testing. An additional 53 samples were absorbed only if agglutination occurred in control tests. Six serums contained nonabsorbable agglutinators. Serums were diluted 1 : 8 and 1 : 16 for testing. These dilutions were selected on the basis of preliminary titrations on each test system.

Serums from 111 chimpanzees were Gm(a+x−b^1−) (Table 2). Forty of these animals were tested for Gm(b^2), Gm(b^3), and Gm(b^4) and were Gm(a+x−b^1−b^2−b^3+b^4+). This combination of Gm factors differs from that observed in three races of man (Table 3). Gm(b^1) has an incidence of 20 to 100 percent in human populations (Table 3), and Gm(b^1+) humans are almost always Gm(b^3+b^4+) (*14*). By contrast, none of the chimpanzees tested were Gm(b^1+), although all were Gm(b^3+b^4+).

The only Gm polymorphism demonstrated was for the factor Gm(c). Thirty-seven of 107 chimpanzees tested were Gm(c+) (Table 2). In man, Gm(c) has been observed primarily in the Negroid race, with an incidence of 30 to 100 percent depending on the Negro population surveyed. A striking difference between the Gm(c) polymorphism in man and chimpanzee is that all Gm(c+) humans are Gm(b^1+), while all Gm(c+) chimpanzees tested were negative for Gm(b^1) (Table 3).

Both Inv(l) and Inv(b), which are present on some human light polypeptide chains, were demonstrable in chimpanzee serums. Twenty-five of 96 chimpanzee serums tested were Inv(l+) (Table 2). This is within the range of frequency for Inv(l+) found in various human population studies (Table 3) (*12*).

Scarcity of reagents for Inv(b) testing limited the number of serums that could be tested for this factor (*15*). Human serums are either Inv(l+b−), Inv(l−b+), or Inv(l+b+), with 90 to 99 percent of serums being Inv(b+). It was assumed that the distribution of Inv factors in chimpanzees might be similar to that in humans. To increase the possibility of identifying Inv(b−) chimpanzees, and in order to do the most critical tests with the smallest amount of available reagents, serums for Inv(b) testing were chosen mainly from Inv(l+) animals. Of 17 Inv(l+) chimpanzee serums, 14 were Inv(b+) and three were Inv(b−) (Table 2). Three Inv(l−) serums were also tested for Inv(b) and all were Inv(b+). Based on these limited observations, it would appear that chimpanzees, like men, are all positive for at least one Inv factor and that most chimpanzees are Inv(b+).

These Gm and Inv data provide evidence for both genetic similarities and differences between man and chimpanzees. The genetic factors detected in chimpanzee serums appear to be antigenically very similar to the corresponding factors detected in human serums. Chimpanzees differ from Caucasian populations in that chimpanzees are 100 percent Gm(a+x−) and demonstrate a polymorphism for

Table 1. Reagents used to determine Gm and Inv factors.

Factor	Agglutinating serum		Antiserum to D*	
	Source	Dilution	Source	Dilution
Gm(a)	Wils	1/8	251	1/5
Gm(x)	Taylor	1/16	Ham	1/10
Gm(b^1)	Draves	1/4	VS	1/5
Gm(b^2)	Davis	1/4	Roehm	1/5
Gm(b^3)	Thomas	1/4	VS	1/5
Gm(b^4)	Burkhart	1/4	VS	1/5
Gm(c)	FPA	1/8	War	1/5
Inv(l)	Math	1/8	Roehm	1/5
Inv(b)	Lucas	1/4	Ham	1/5

* Rh-positive cells.

Table 2. Summary of results of Gm and Inv testing on chimpanzee serums. In the categories where polymorphism was demonstrated, an analysis of the distribution is shown.

Number tested	Number positive								
	Gm							Inv	
	a	x	b^1	b^2	b^3	b^4	c	l	b
111	111	0	0						
40				0	40	39*			
107							37		
96								25	
71							19	0	
25							14	25	
20									17
17							11	17	14
3							0	0	3

* One nonabsorable agglutinator.

Table 3. Summary of the approximate frequencies (percentages) of Gm and Inv factors in chimpanzees and various races of man.

Population*	Gm factors							Inv factors	
	a	x	b^1	b^2	b^3	b^4	c	l	b
White (U.S.)	57	20	90	90	90	90	0	20	99
Negroid (African)	100	0	100	0	90	95	30–100	50	90
Mongoloid (Japanese)	100	30	20	20	60	20	0	50	88
Chimpanzee	100	0	0	0	100	100	35	25	?

* The data on White, Negroid, and Mongoloid populations are taken from Steinberg and co-workers (*12, 14, 18*).

Gm(c). Chimpanzees differ from Negro populations in being 100 percent Gm(b^1—).

A further similarity between the Gm factors of man and chimpanzee is their mode of inheritance. In man, Gm factors are inherited as dominants or codominants (*10*). Studies of four chimpanzee families containing both parents and one or more offspring indicate that Gm(c), the only Gm factor showing polymorphism in the chimpanzee is inherited as a dominant in this species.

The amount of γ-globulin as determined by the zinc turbidity method (*16*) in 64 of the chimpanzee serum samples ranged from 0.64 to 1.38 g/100 ml. This compares with the range of 0.71 to 1.3 g/100 ml for normal human serums. No correlation was observed between the amounts of IgG and Gm or Inv types. The absence of Gm(b^1), and Gm(b^2), and Gm(x) and the variable presence of Gm(c), Inv(l) and Inv(b) in the chimpanzee do not merely reflect differences in serum γ-globulin concentrations.

Chimpanzee serums were tested by Ouchterlony analysis with monkey antiserums specific for human γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins (*2*). All serums tested gave precipitin bands with each antiserum. Similarly, Ouchterlony tests with rabbit antiserums specific for either κ- or λ-chains were positive for all tested serums. Hence, chimpanzee serums contain molecules antigenically related to human γ_{2a}-, γ_{2b}-, and γ_{2c}-globulins, and also to human κ- and λ-chains.

Thus chimpanzee serums contain at least four of the human Gm factors and two Inv factors. They also contain molecules antigenically related to those heavy and light polypeptide chains of human IgG that seem to be necessary substrates for the expression of Gm and Inv factors. Whole serum was used in these experiments, and it will be important in the future to isolate immunoglobulins from chimpanzee serums and examine genetic and antigenic characteristics of the isolated proteins. In addition, since there may be subspecies or races of chimpanzees (*17*), a more complete understanding of primate immunoglobulin genetics will probably require investigation of subspecies of chimpanzees, as well as of other nonhuman primates.

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Active Transport of 5,5-Dimethyl-2,4-Oxazolidinedione

Abstract. *5,5-Dimethyl-2,4-oxazolidinedinedione, a substance commonly used to estimate intracellular* p*H, moves against both a concentration gradient and a hydrogen-ion gradient in the everted gut sac. Furthermore, the value of the flux ratio for this substance under conditions of zero electrochemical potential across the bowel wall unequivocally demonstrates active transport.*

In recent years partitioning of the weak acid 5,5-dimethyl-2,4-oxazolidinedione (DMO) between the intra- and extracellular fluid has been used to estimate intracellular *p*H (*1*). Implicit in the method are the essential assumptions (i) that the acid is not bound to intra- or extracellular proteins, (ii) that its disassociation constant (pK_a) is the same inside and outside the cell, (iii) that, essentially, it is not metabolized, and finally (iv) that it is passively distributed between the intra- and extracellular fluid in accordance with the hydrogen-ion gradient across the cell membrane. If any one of these assumptions is incorrect in any biologic system, then the uncritical use of DMO in other systems may be invalid. While several studies have shown that DMO is neither metabolized nor bound to protein (*2*), the possibility that it is actively transported in mammalian tissue has never been carefully investigated. Should there exist, for example, a carrier mechanism in the plasma membrane for the transport of DMO into the cell against an electrochemical gradient, one would expect an intracellular concentration higher than that appropriate for the actual H^+ gradient; hence the calculated value for the intracellular *p*H would be erroneously high. The small intestine of the rat, a tissue that actively transports a number of substances, was used in our investigation because experimental methods were available which provided rigid control of transmembrane potential differences as well as concentration and *p*H gradients.

Sprague-Dawley female rats (240 to 260 g) were given free access to rat chow and water before they were killed by decapitation. The entire small bowel was excised, flushed of its contents with cold saline, and cut into ten segments of equal length. For purposes of identification these segments were numbered 1 through 10, from the