PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 307–310 (1975)

Immunoglobulin Allotypes in Symptomatic and Asymptomatic Pigeon Breeders[1] (38795)

VERNON L. MOORE, MOSES S. SCHANFIELD, JORDAN N. FINK, AND H. HUGH FUDENBERG[2]
(Introduced by Joseph J. Barboriak)

Allergy Section, Department of Medicine, The Medical College of Wisconsin and the Research Service, Wood Veterans Administration Hospital, Milwaukee, Wisconsin 53193 and Section of Immunology, University of California, San Francisco, California 94143

Pigeon breeder's disease (PBD) is a hypersensitivity pneumonitis that probably results from continued exposure to pigeon-associated antigens, e.g., pigeon droppings, serum, feathers, egg white and yolk (1–3). In most cases, large quantities of specific antibodies are detectable in the serum of patients with this disease (4–6). However, nearly 40% of asymptomatic pigeon breeders also develop detectable circulating antibodies to pigeon antigens, albeit of lower titers (7). Furthermore, recent studies have indicated that symptomatic breeders displayed cell-mediated hypersensitivity (CMH) to pigeon antigens, while asymptomatic breeders failed to show CMH (6, 8).

Another striking observation with regard to this hypersensitivity pneumonitis is that the incidence of pigeon breeder's disease in individuals, presumably exposed to similar antigenic "loads" for long periods of time, is only 6% (8). One explanation of this low incidence may be that ill breeders are genetically predisposed to develop high titers of circulating antibody, as well as cell-mediated hypersensitivity to pigeon antigens. One method of studying this aspect of the disease is immunoglobulin typing, since individuals with the haplotype $Gm^{a;g}$ produce higher titers of antibody to *Salmonella adelaide* flagellar antigens (9). Furthermore, certain allotypes seem to be associated with the serum concentration of different IgG subclasses (10–14).

We therefore studied the Gm allotypes of a group of symptomatic breeders, as well as of a group of similarly exposed but asymptomatic breeders.

Materials and Methods. Blood was collected from patients known or suspected of having pigeon breeders' disease. The diagnosis of PBD is based upon the criteria indicated previously (2).

Immunoglobulin allotyping was done on microtiter plates using a passive hemagglutination inhibition system (15, 16). All sera were tested at a dilution of 1:20, with appropriate controls. Any serum with demonstrable agglutinating activity was heat inactivated at 65° for 10 min and retyped. The reagents used are presented in Table I. Following the recommendations of the WHO Workshop on Human Immunoglobulin Allotypes (July 16–19, 1974) only the alphameric notation of the two notations recommended will be used (Table I).

Results. The results of immunoglobulin typing of the serum of ill pigeon breeders are shown in Table II, and of serum from asymptomatic breeders in Table III. There were nine adult male Caucasian symptomatic breeders tested. Two were Gm(f, z, a; b, g, v), three were Gm(f, z, a, x; b, g, v) and four were Gm(f; b, v). None were of the phenotypes Gm(z, a; g, v) or Gm(z, a, x; g, v).

Of the 14 asymptomatic breeders tested, one was an adult Caucasian female (G. G.), three were juvenile Caucasian males (Jo. K., M. K., and P. K.), ranging in age from 12–16 yr and 10 were adult Caucasian males. Four were of the phenotype Gm(f, z, a; b, g, v), three were Gm(f, z, a, x; b, g, v),

[1] Supported by funds from Specialized Center of Research Grant No. (SCOR) HL15389 from the National Heart and Lung Institute, a grant in memory of William Heller, Sr., NIH Postdoctoral Research Fellowship, No. H052597-02, and grants from the National Institutes of Health (AI-09145 and HD-05894).

[2] Present address: Dept. of Basic and Clinical Microbiology, Medical University of of South Carolina, Charleston, SC 29401.

TABLE I. REAGENTS USED FOR IMMUNOGLOBULIN ALLOTYPING.[a]

Allotype designation			
Alphameric	Numeric	Agglutinator	Coat
G1m a	1	Hel or Pan	Dwi
x	2	Dev	Yap
f	3	Sta	Dan
z	17	Pon	Dwi
G3m b0	11	Har	Hun
b1	5	Ble	Hun
b3	13	Log	Hun
b5	10	Ste	Hun
c3	6	And	1872A
c3	6	Alf	1872A
c5	24	Hod	1872A
g	21	B755[b]	Sul
g	21	Gha	Sul
g	21	Leh	Sul
s	15	Gai	Puh
t	16	Ros	Puh
v	27	Joh	Sul
A2m 1	1	Far	Her
2	2	Tay	For
Km (Inv) 1	1	Cla	Abr
1	1	Sim	Abr

[a] Recommended nomenclature; WHO Workshop on Human Immunoglobulin Allotypes, Rouen, France, July 16–19, 1974.

[b] Generously provided by Dr. S. B. Litwan.

seven were Gm(f; b, v) and one was Gm(z, a; g, v).

All individuals were A2m (1) positive and A2m (2) negative with the exception of a single individual (A. Z.). This individual probably has the haplotype $Gm^{z,\ a,\ x;\ g,\ v}$ $A2m^2$. All other individuals had combinations of the following haplotypes: $Gm^{z,\ a;\ g,\ v}$ $A2m^1$, $Gm^{z,\ a,\ x;\ g,\ v}$ $A2m^1$ and $Gm^{f;\ b,\ v}$ $A2m^1$. Due to overall uniformity of the $A2m^1$ haplotype it will not be discussed further.

With the exception of a single individual (G. H.), all individuals studied were *Km*(Inv) (1) negative.

Discussion. This study has shown that there is no association between Gm allotypes and the presence of hypersensitivity pneumonitis in the pigeon breeders tested. Forty-four percent of the symptomatic patients studied were homozygous $Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$, while the remainder were heterozygous for either $Gm^{f;\ b,\ v}/Gm^{z,\ a;\ g,\ v}$ or $Gm^{f;\ b,\ v}/Gm^{z,\ a,\ x;\ g,\ v}$. Interestingly, none of thc ill breeders studied were homozygous for $Gm^{z,\ a;\ g,\ v}$, the genotype associated with increased antibody production against *S. adelaide* flagellar antigen (9).

In the asymptomatic group, 8 of 14 were homozygous; of these 7 had the genotype $Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$ and 1 had the genotype $Gm^{z,\ a;\ g,\ v}/Gm^{z,\ a;\ g,\ v}$. This individual did not have detectable precipitating antibodies to pigeon serum or pigeon droppings. Since this individual had been exposed to pigeon antigens for several years, this suggests that the genotype $Gm^{z,\ a;\ g,\ v}/Gm^{z,\ a;\ g,\ v}$ is not associated with the production of large quantities of antibodies to pigeon antigens, as has been observed with flagellar antigens (9).

The individuals studied have been exposed to pigeon antigens daily for several years. Presumably, individuals in both the symptomatic and asymptomatic groups have been exposed to similar antigenic "loads". Since the incidence of hypersensitivity pneumonitis is about 6% (8), it appears that antigen inhalation is a necessary but not sufficient factor for the induction of the disease; the additional factors are as yet unknown.

While the pathogenetic mechanism(s) of hypersensitivity pneumonitis have not been fully elucidated, both cellular and humoral immunologic mechanisms may contribute to the disease (6, 8, 17). Qualitative and quantitative differences in cell-mediated immunologic responses related to *Gm* allotypes would seem to be unlikely. It is, however, probable that cell-mediated immunologic responses in man are related to the HLA- or MLC-related Ir systems. The relative importance of this system in hypersensitivity pneumonitis remains to be determined.

In summary, genetic studies on Gm allotypes in symptomatic and asymptomatic pigeon breeders indicate that there are no correlations between the Gm allotypes and the presence of this disease.

Summary. Comparison of immunoglobulin allotypes were studied in a group of patients with pigeon breeder's disease and in similarly exposed but asymptomatic indi-

TABLE II. IMMUNOGLOBULIN ALLOTYPES IN SYMPTOMATIC PIGEON BREEDERS.[a, b]

Subject	G1m	G3m	A2m	Probable Haplotypes
P. A.	f, z, a, x	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a,\ x;\ g,\ v}$
W. C.	f, z, a	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a;\ g,\ v}$
C. F.	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
G. H.	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
R. K.[c]	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
J. K.	f, z, a, x	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a,\ x;\ g,\ v}$
A. R.	f, z, a	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a;\ g,\ v}$
E. S.	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
H. S.	f, z, a, x	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a,\ x;\ g,\ v}$

[a] All individuals reported to be G3m (b) positive are positive for G3m (b0, b1, b3 and b5).
[b] All haplotypes include $A2m^1$.
[c] Father of Ja. K., Jo. K., M. K. and P. K. (Table III).

TABLE III. IMMUNOGLOBULIN ALLOTYPES IN ASYMPTOMATIC PIGEON BREEDERS[a, b]

Subject	G1m	G3m	A2m	Probable Haplotypes
Ja. K.[c]	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
Jo. K.[c]	f, z, a	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a;\ g,\ v}$
M. K.[c]	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
P. K.[c]	f, z, a	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a;\ g,\ v}$
T. K.	f, z, a	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a;\ g,\ v}$
F. A.	f, z, a, x	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a,\ x;\ g,\ v}$
G. G.	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
R. G.	f, z, a	b, g, v	1	$Gm^{f;\ b,\ v}/Gm^{z,\ a;\ g,\ v}$
E. J.	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
G. P.	z, a	g, v	1	$Gm^{z,\ a;\ g,\ v}/Gm^{z,\ a;\ g,\ v}$
R. T.	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
J. W.	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$
A. Z.	f, z, a, x	b, g, v	1, 2	$Gm^{f;\ b,\ v}/Gm^{z,\ a,\ x;\ g,\ v^d}$
E. G.	f	b, v	1	$Gm^{f;\ b,\ v}/Gm^{f;\ b,\ v}$

[a] All individuals reported to be G3m(b) positive are positive for G3m(b0, b1, b3 and b5).
[b] All halotypes include $A2m^1$ except that identified with four asterisks (****).
[c] Sons of symptomatic breeder R. K. (Table II).
[d] Probable haplotype $Gm^{z,\ a,\ x;\ g,\ v}A2m^2$.

viduals. The study revealed that the disease is not correlated with immunoglobulin allotypes. Furthermore, the phenotype *Gm*(a; g) was not associated with high levels of serum antibodies to pigeon antigens.

1. Fink, J. N., Barboriak, J. J., and Sosman, A. J., J. Allergy **39**, 214 (1967).
2. Fink, J. N., Sosman, A. J., Barboriak, J. J., Schlueter, D. P., and Holmes, R. A., Ann. Intern. Med. **68**, 1205 (1968).
3. Reed, C. E., Sosman, A. J., and Barbee, R. A., J. Amer. Med. Ass. **193**, 261 (1965).
4. Fink, J. N., Barboriak, J. J., Sosman, A. J., Bukosky, R. J., and Arkins, J. A., J. Lab. Clin. Med. **71**, 20 (1968).
5. Fink, J. N., Tebo, T., and Barboriak, J. J., J. Immunol. **103**, 244 (1969).
6. Moore, V. L., Fink, J. N., Barboriak, J. J., Ruff, L. L. and Schlueter, D. P. J. Allergy Clin. Immunol. **53**, 319 (1974).
7. Barboriak, J. J., Sosman, A. J., and Reed, C. E., J. Lab. Clin. Med. **65**, 600 (1965).
8. Caldwell, J. R., Pearce, D. E., Spencer, C., Leder, R., and Waldman, R. H. J. Allergy Clin. Immunol. **52**, 225 (1973).
9. Wells, J. V., Fudenberg, H. H., and Mackay, I. R., J. Immunol. **107**, 1505 (1971).
10. Yount, W. J., Kunkel, H. G., and Litwin, S. D., J. Exp. Med. **125**, 177 (1967).
11. Litwin, S. D., and Balaban, S., J. Immunol. **108**, 991 (1972).

12. Morrell, A., Skvaril, F., Steinberg, A. G., van Loghem, E., and Terry, W. D., J. Immunol. **108,** 195 (1972).
13. Steinberg, A. G., Morrell, A., Skvaril, F., and van Loghem, E., J. Immunol. **110,** 1642 (1973).
14. Van der Geissen, M., Freyee, W., Rossouw, E., and van Loghem, E., Clin. Exp. Immunol. **14,** 127 (1973).
15. Schanfield, M. S., Sebring, E. B., and Polesky, H. F., American Society of Clinical Pathology, Workshop Manual Paternity Testing (1973).
16. Schanfield, M. S., and Fudenberg, H. H., "Bio-social Interrelations in Population Adaption", Mouton, the Hague, Netherlands (in press).
17. Hargreave, F. E., and Pepys, J., J. Allergy Clin. Immunol. **50,** 127 (1972).

Received December 11, 1974. P.S.E.B.M. 1975, Vol. 149.