MEASLES-NEUTRALIZING AND HI ANTIBODY TITERS OF HUMAN GAMMA GLOBULIN PREPARATIONS

Stephen J. Millian, Ph.D.
Morris Schaeffer, M.D.

GAMMA GLOBULIN has been used extensively as a prophylactic agent to protect individuals exposed to poliomyelitis, infectious hepatitis, and measles or to modify the effects of measles. The current use of an attenuated measles vaccine has introduced an additional need for gamma globulin and necessitated the standardization of the preparation with regard to measles antibody. Heretofore, it has been possible for gamma globulin to be standardized, primarily with respect to its poliomyelitis antibody titer (*1*). The establishment of potencies for the other viral components has been precluded because of the lack of a serologic test for hepatitis and, until recently, for measles. However, suitable serologic tests for measles are now available (*2–4*).

The anticipated extensive use of measles vaccine in conjunction with gamma globulin prompted us to determine whether significant variations in measles antibody levels exist in commercial gamma globulin preparations labeled "poliomyelitis immune globulin (human)" and to determine whether these preparations meet the newly promulgated specifications (*5*).

Materials and Methods

Sixteen different lots of commercial gamma globulin, distributed over a period of several years, were tested. These had been prepared from pooled normal human plasma and were purchased on the open market for health department use. The globulins were stored at 4° centigrade until tested simultaneously with reference antiserum.

The reference measles antiserum was obtained from the Division of Biologic Standards of the National Institutes of Health, Public Health Service, and used as a standard for comparing the measles antibody titers of the gamma globulin preparations.

Measles hemagglutination inhibition (HI) tests were performed by adapting Rosen's method (*3*) to a microtitrator technique with only one modification which involved the use of 1 percent monkey erythrocytes. The HI end points were derived from two separate tests performed in duplicate and were the highest dilutions of the gamma globulin preparations which completely inhibited hemagglutination of four hemagglutinating (HA) units of virus.

The measles virus HA antigen and the normal tissue control materials were obtained from Microbiological Associates, Bethesda, Md. The measles HA antigen had a titer of 1:32.

Measles-neutralizing antibody levels were determined in accordance with the method specified by the Public Health Service (*5*) using 100 $TCID_{50}$ per 0.1 ml. of measles virus and a 0.1 ml. aliquot of a serum dilution. End points were calculated according to the method of Reed and Muench (*6*) and reported as reciprocals of initial serum dilutions.

Results and Discussion

The measles-neutralizing and hemagglutination-inhibiting antibody titers of the 16 lots of gamma globulin evaluated are listed in the table.

Dr. Millian is chief of the virus unit, and Dr. Schaeffer is director, bureau of laboratories, New York City Department of Health.

The preparations were found to have uniform antibody levels as the titer variations were less than fourfold, which is within the limit of error of this test. The gamma globulin lots had an arithmetic neutralizing antibody mean titer of 1:1,640 and a mode of 1:1,800, and an arithmetic HI antibody mean titer of 1:352 and a mode of 1:256. In a similar study made elsewhere (7) with 23 lots of gamma globulin prepared from placental extract, the measles-neutralizing antibody titers were found to range from 1:500 to 1:2,000 with a mean titer of 1:1,500 and a mode of 1:2,000. These data are in agreement with our findings and indicate that measles-neutralizing antibodies, like poliomyelitis antibodies, occur in comparable titer in placental extract and venous blood.

In the two instances when more than one lot of gamma globulin from a single pharmaceutical house were available for testing, the neutralizing and HI titers were consistent for each processor. It may be of interest to note that the three preparations from vendor C had neutralizing titers lower than the mean titer, while 8 of the 10 preparations from vendor S were greater than the mean. In addition, two of the three preparations from vendor C had HI titers of less than the mean, while all 10 globulins tested from vendor S had titers in excess of the mean.

Although eight of the biologics were 1 to 7 years past their expiration dates when tested, it is significant that their neutralizing and HI antibody levels were found to be equal to preparations with unexpired dates.

In every instance the neutralization titers of the globulin preparations were approximately two to three times the value of the measles reference antiserum, which had titers of 700, 750, and 700 on three separate occasions. It is interesting to note that the minimum specifications for measles-immune globulin require that "each lot of final product shall contain a measles antibody level of 0.5 times the level of the NIH reference measles serum" and that "a plus or minus variation of one twofold dilution is acceptable." While even outdated gamma globulin preparations, as currently prepared, satisfy the minimal stipulated potency requirements, most of the preparations had titers in excess of the acceptable potency requirements. Such gamma globulin preparations containing excessive amounts of measles-neutralizing antibody may be inimical to and actually preclude measles immunization when live virus is employed.

Measles-neutralizing and hemagglutination-inhibiting antibody titers of human gamma globulin preparations

Vendor and expiration date	Measles antibody titer [1]	
	Neutralizing	Hemagglutination inhibiting
S. July 10, 1956	1,400	256
S. May 10, 1957	1,800	512
D. Sept. 5, 1962	1,800	512
S. Apr. 4, 1963	1,500	512
S. Sept. 6, 1963	1,800	512
C. Aug. 25, 1963	1,300	128
C. Sept. 26, 1963	1,100	128
C. Nov. 10, 1963	1,400	256
H. May 1, 1964	1,300	256
S. May 14, 1964	1,900	256
S. Aug. 7, 1964	2,100	256
S. Sept. 25, 1964	1,700	512
S. Nov. 27, 1964	1,800	256
S. Jan. 30, 1965	1,800	256
S. Feb. 12, 1965	1,700	512
M. May 2, 1965	1,900	512

[1] Reciprocal of dilution.

Summary

Sixteen lots of commercial poliomyelitis-immune human gamma globulin were titrated for measles-neutralizing and hemagglutination-inhibiting antibodies. The neutralizing antibody titers of the various lots ranged from 1:1,100 to 1:2,100 and were approximately two to three times the potency of a reference measles serum. The hemagglutination-inhibiting antibody titers ranged from 1:128 to 1:512.

Gamma globulin preparations tested 1 to 7 years after their expiration dates had neutralizing and HI titers comparable to unexpired preparations.

REFERENCES

(1) U.S. Public Health Service: Minimum requirements: Immune serum globulin (human). Regulations, 3d revision. U.S. Government Printing Office, Washington, D.C., 1953.

(2) Enders, J. P., and Peebles, T. C.: Propagation in tissue cultures of cytopathogenic agents from

patients with measles. Proc Soc Exp Biol Med 86:277 (1954).
(3) Rosen, L.: Hemagglutination and hemagglutination-inhibition with measles virus. Virology 13:139 (1961).
(4) Rosanoff, E. I.: Hemagglutination and hemadsorption of measles virus. Proc Soc Exp Biol Med 106: 563 (1961).
(5) U.S. Public Health Service: Biological products; regulations. Additional standards: Measles immune globulin (human). PHS Publication No. 437. U.S. Government Printing Office, Washington, D.C., 1963.
(6) Reed, L. J., and Muench, H.: A simple method of estimating fifty percent endpoints. Amer J Hyg 27:493 (1938).
(7) Cabasso, V. J., Kiser, K. H., Clarke, F. H., and Stebbins, M. R.: Measles and poliovirus antibodies in commercial preparations of human gamma globulin. J Immun 90:81 (1963).

Safer Dental X-ray Machines

Today 75,000 X-ray machines in U.S. dentists' offices meet recommended standards, in comparison with only 25,000 in 1960. Surgeon General Luther L. Terry attributes the increase to continuing inspection and correction programs conducted by State health agencies in cooperation with State dental societies and the Public Health Service.

Of the more than 200,000 X-ray machines in the United States, approximately 100,000 are owned and operated by dentists, who expose 185,000,000 dental films a year.

The Surgeon General reported that two survey techniques increasingly employed by public health agencies were responsible for much of the improvement. A mail procedure called Surpak, developed by the Division of Radiological Health, Public Health Service, enables a rapid survey to be made of dental X-ray machines. The Surpak kit includes a photographic film in a sealed envelope which the dentist places under the pointer cone of his X-ray machine, exposes according to directions, and sends to his State or local health agency, which forwards it to the Public Health Service for development and analysis. The Service then sends any filters and collimators needed to the State for redistribution to the dentist, together with instructions for installation. More than 58,000 Surpaks have been distributed by the Public Health Service to 39 States. A more comprehensive method of survey is an on-site inspection of dental offices, usually covering equipment, technique, and occupational exposure.

The Public Health Service is also now developing a procedure similar to the dental Surpak for testing X-ray units in physicians' offices.