

OPSONIC ACTIVITY OF AGAMMAGLOBULINEMIA SERUM DETERMINED BY OXYGEN CONSUMPTION CAPACITY OF LEUKOCYTES*

By

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(Received June 20, 1981)

ABSTRACT

In evaluating the therapeutic efficacy of gammaglobulin preparations in patients with agammaglobulinemia, opsonic activity of patients' sera in terms of oxygen consumption capacity of normal leukocytes is advocated as a useful index. Although the serum IgG level is a commonly used indicator, its determination alone was found out not always to reflect antibody levels in such sera. The method described here is simple and gives quantitative results and deserves a wider trial in controlling gammaglobulin replacement therapy.

INTRODUCTION

Easy susceptibility to infections in patients with a- and hypogammaglobulinemia is attributed to a lack of antibodies in their sera, and regular administration of gammaglobulin preparations is therefore indicated to provide them with sufficient humoral factors to counteract invading organisms. Although serum immunoglobulin levels have generally been used as indicators in regulating replacement therapy, no specific index is currently in use in evaluating its actual efficacy. In this study, the opsonic activity of patients' sera before and after gammaglobulin therapy was determined by measuring the oxygen consumption capacity of leukocytes. This method is recommended as one of the objective measures of controlling gammaglobulin therapy in such patients.

SUBJECTS AND METHODS

Three patients with agammaglobulinemia were examined: patient 1, a 2-year-old girl with the sporadic type of agammaglobulinemia combined with vaccine-associated poliomyelitis¹⁾; patient 2, a 6-year-old boy and patient 3, a 4-year-old boy, both with possible X-linked recessive type. By the time of this study, they had been given regularly intravenous gammaglobulin preparations (S-sulfonated human gammaglobulin²⁾ in patients 1 and 2 and pepsin-treated preparations in patient 3), when examined without overt infections (patients 1 and 2) or with acute pharyngitis (patient 3).

Sera were separated from blood specimens obtained before and after an intravenous gammaglobulin administration and assayed on the day of bleeding or stored at -80°C until use.

* 小林陽之助, 坂野堯, 林和成, 臼井朋包: 好中球酸素消費能により測定した無ガンマグロブリン血症血清のオプソニン活性

Informed consent was obtained from the parents. Gammaglobulin preparations were initially adjusted with saline to give 1,250 mg/dl of IgG and further with HEPES buffer (17 mM N-hydroxyethylpiperazine-N'-ethane sulfonic acid, pH 7.4, 120 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄ and 5 mM glucose) to indicated final concentrations as serum's in reaction mixtures. Immunoglobulins and complement components were determined by a single radial immunodiffusion method and CH50 by the method of Meyer⁹⁾.

Healthy adult volunteers donated heparinized venous blood, from which polymorphonuclear leukocytes (PMNL) were isolated by 3% dextran sedimentation, Ficoll-sodium metrizoate gradient centrifugation and hypotonic lysis of residual erythrocytes. The purity and viability of prepared cells were higher than 95%. The blood type of the patients and the PMNL donors were matched in terms of the ABO blood group system.

Four kinds of phagocytizable particles were tested. Zymosan A from *S. cerevisiae* (Sigma Chem. Co.) was washed and suspended in HEPES buffer at a concentration of 0.6 mg/10⁶ PMNL. Three species of bacteria, i. e., *Staphylococcus aureus* 209P (*S. aureus*), *Strepto-*

coccus faecalis (*S. faecalis*) and *Escherichia coli* NIHJ-JC2 (*E. coli*), were cultured in brain heart infusion broth for 18 hours. After they had been washed and standardized (5 × 10⁹ bacteria/ml), they were heat-killed and used at a bacteria/PMNL ratio of 100.

Oxygen consumption of PMNL was determined with a Clark type oxygen electrode (Yellow Springs Instrument Co.) by the method of Nakamura et al.⁴⁾ and expressed as n moles of oxygen consumed/min/10⁶ PMNL. A reaction mixture of 1.0 ml contained PMNL (2 × 10⁶/ml) and the serum or gammaglobulin preparation to be tested in a concentration of 10% for zymosan, 5% for *S. aureus*, 5% for *S. faecalis* or 10% for *E. coli* added as particles to be opsonized. Heat-inactivation of sera was at 56°C for 30min.

RESULTS

Preliminary studies showed that none of the unopsonized particles was ingested by PMNL and that oxygen consuming PMNLs were confirmed morphologically to have ingested particles. Table shows the results of opsonic activity expressed in amounts of oxygen consumed by PMNLs. As is clear from the Table, all gammaglobulin preparations were completely

Table Opsonic activity of agammaglobulinemia sera and gammaglobulin preparations in terms of oxygen consumption capacity of leukocytes

	Before*	Patient 1 GGP**	After*	Before	Patient 2 GGP	After	Patient 3 19D after***	Normal range
Zymosan	5.9#	0	3.9	9.7	0	8.9	6.6	7.1 ± 2.3##
<i>S. aureus</i>	6.2	1.1	6.2	4.0	1.2	5.3	0.8	4.1 ± 0.9
<i>S. faecalis</i>	0	0	0	0	0	0	0	2.7 ± 0.6
<i>E. coli</i>	1.5	1.3	3.0	1.2	0.5	1.3	0.6	1.6 ± 0.3
IgG (mg/dl)	420	(1,250)	1,050	84	(1,250)	462	100	
CH50 (u/ml)	25	nd###	5>	36	nd	5>	nd	30- 40
C1q (%)	79	0	79	59	0	53	nd	100
C3(mg/dl)	83	10>	92	73	10>	78	75	60-130
C4 (mg/dl)	55	1>	55	53	1>	51	48	20- 40
C5 (%)	103	0	122	96	0	81	89	100

*: Serum specimen obtained before and just after an intravenous injection of a gammaglobulin preparation (2.5 g)

** : GGP (Gammaglobulin preparation)

*** : Serum specimen obtained 19 days after the last injection of GGP

: n moles of oxygen consumed/min/10⁶ PMNL.

: Mean ± 1 SD (n=20 adults)

: not done

devoid of opsonic activity for both zymosan and *S. faecalis*. Opsonic activity for zymosan, which was normal in pre-injection samples, was consistently reduced in the specimens obtained just after the injection (patients 1 and 2). This finding can be explained by the activation of the complement system, in which CH50, although grossly normal in the pre-injection sera, fell below detectable levels in the post-injection sera. C3 and C5 levels remained unchanged within the normal range with constantly elevated C4 values in all three patients. Incubation of normal as well as patients' sera with each gammaglobulin preparation at 37°C for 30 min also reduced the opsonic activity for zymosan (Data not shown). Heating of sera almost completely abolished the opsonic activity for zymosan. With *S. aureus* as phagocytizable particles, the sera of patients 1 and 2 retained normal activity, while patient 3 showed a marked decrease, although IgG levels were grossly comparable between patients 2 and 3. An additive effect observed in patient 2 was not seen in patient 1, and vice versa with *E. coli*. Heat inactivation of sera resulted in a loss of activity by 50–80%. Opsonic activity for *S. faecalis* was absent in all three patients. Heating of normal serum was associated with an almost complete loss of activity, but the addition of cord serum, which is lacking in opsonic activity by itself but serves as a complement source⁵⁾, restored normal activity. Its deficiency in patients' sera in spite of the presence of complement appears to indicate that this defect is inherent in these patients. Opsonic activity for *E. coli* was normal in patients 1 and 2 but was reduced in patient 3 as for *S. aureus*. No appreciable loss of opsonic activity for *E. coli* was followed by heat inactivation of serum.

DISCUSSION

Administration of gammaglobulin preparations is indispensable in the treatment of patients with antibody deficiency syndromes and is generally intended to produce a serum level of 300 mg/dl of IgG⁶⁾. Although the clinical evaluation of patients is of prime significance, an objective means by which to assess its therapeutic efficacy appears to be to find out whether a sufficient supply of the specific antibody which the patient is actually lacking can

be obtained by such therapy. For this purpose, the opsonic activity expressed in terms of oxygen consumption capacity of leukocytes was evaluated in this study and turned out to represent an important aspect of the opsonization phenomenon. Efficient opsonization is known to be a prerequisite for subsequent attachment to and ingestion by leukocytes, as a trigger of their oxidative metabolism. Oxygen consumption is the initial event in this respiratory burst⁷⁾, and consequently its determination should indicate the presence and extent of opsonins when intact leukocytes and particles to be opsonized are available. Furthermore, the current method is simple and yields quantitative results, precluding other cumbersome methods, such as counting of the ingested particles.

Complete absence of opsonic activity for zymosan as well as for *S. faecalis* is likely to be due to a lack of complement in these gammaglobulin preparations, which, however, are potent sources of antibodies against *S. aureus* and *E. coli*. Their additive enhancing effects are unpredictable, differing from one particle to another or from one patient to another. Inconsistencies in this supplementary effect, if they can be regarded as such, will have to be clarified by the accumulation of more cases. One observation which deserves further comment is that an IgG level alone is not to be relied on as a therapeutic guide. Although IgG levels are comparable between patients 2 and 3, the latter exhibited a marked decrease of bacterial particles, while the serum of the former patient showed a normal capacity to opsonize. In this context, one more mention should be made of patients 1 and 2, between whom, in spite of a five-fold difference in IgG levels, opsonic activities are almost equal. For such a comparison, however, the difference in gammaglobulin preparation should be taken into consideration, i.e., S-sulfonated in patients 1 and 2 and pepsin-treated in patient 3.

In applying this method to clinical use, one of the major considerations is the choice of phagocytizable particles. As patients are exposed to the potential hazards of numerous microorganisms, a battery of only four particles may be incomplete. However, as far as heat stability is concerned, as their opsonic requirement covers heat-labile (zymosan), heat-stable (*E. coli*) or both opsonins (*S. aureus* and *S.*

faecalis), results based on such diverse particles will be sufficient to make a general estimate on the opsonic ability of the sera of patients as well as of gammaglobulin preparations.

According to Fukumoto et al.⁸⁾, S-sulfonated human gammaglobulin preparation reduces only the first component of the complement without any reduction of other components and consequently with only a minimal depression of CH50. The marked reduction in complement activity noted in patients 1 and 2 is still unexplained. Although the Clq level, determined by an immunochemical method, remained unchanged in the present study, there is a possibility that its activity may have been reduced by gammaglobulin preparations.

Although the small size of the patient sample studied here prevents extrapolation of the results to the opsonic activity of agammaglobulinemic sera in general, the method used will certainly be a valuable tool in monitoring gammaglobulin replacement therapy in patients with antibody deficiency syndrome.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid from the Japan Medical Research Foundation and Grants-in-Aid (Projects NO. 157253 and 544050) from the Ministry of Education, Science and Culture of Japan.

REFERENCES

- 1) Sakano, T., Kittaka, E., Tanaka, Y., Yamaoka, H., Kobayashi, Y. and Usui, T.: Vaccine-associated poliomyelitis in an infant with agammaglobulinemia, *Acta Paediatr Scand*, 69, 549, 1980.
- 2) Masuho, Y., Tomibe, K., Matsuzawa, K. and Ohtsu, A.: Development of an intravenous γ -globulin with Fc activities, I, Preparation and characteristics of S-sulfonated human γ -globulin, *Vox Sang*, 32, 175, 1977.
- 3) Meyer, M. M.: *Experimental Immunochemistry*, Kabat, E. A. and Meyer, M. M., editors, Springfield, Ill, 1961, Thomas, pp. 133-240, 2nd ed.
- 4) Nakamura, M., Nakamura M., Okamura, J. and Kobayashi, Y.: A rapid and quantitative assay of phagocytosis-connected oxygen consumption by leukocytes in whole blood, *J Lab Clin Med.*, 91, 568, 1978.
- 5) Kobayashi, Y. and Usui, T.: An opsonic activity of cord serum—An evaluation based on determination of oxygen consumption by leukocytes, *Pediatr Res* (In press).
- 6) Hong, R.: *Nelson Textbook of Pediatrics*, Vaughan, V. C., III, McKay R. J., Jr. and Behrman R. E., editors, Philadelphia, 1979, M. B. Saunders, pp. 582-598, 11th ed.
- 7) Sbarra, A. J. and Karnovsky, M. L.: The biochemical basis of phagocytosis I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes, *J Biol Chem.*, 234, 1355, 1959.
- 8) Fukumoto, Y., Inai, S., Nagaki, K., Iida, K., Yamaguchi, E., Masuho, Y. and Watanabe, T.: Interaction of S-sulfonated human IgG with human complement and its components, *J Biochem.*, 82, 955, 1977.