Acta Medica Okayama

Volume 22, *Issue* 2 1968 *Article* 4

APRIL 1968

Suppression of antibody formation by the RES-blockade. II. Effects of the RES-blockade with radioactive gold colloid

Shinji Ohbuchi*

*Okayama University,

Suppression of antibody formation by the RES-blockade. II. Effects of the RES-blockade with radioactive gold colloid*

Shinji Ohbuchi

Abstract

For the purpose of revealing the interaction between macrophages and plasma cells in relation to antibody formation and information for cell specialization, the proliferation of plasma cell by antigenic stimulation was observed in the rats whose RES had been previously injured by radiogold. The production of the circulating antibody was markedly suppressed by the pretreatment with radiogold. Histological observation revealed that the plasma cells and lymphocytes were completely obliterated and the tissues were replaced by the basophilic cells and fibroblastic cells. Lymph nodes which contained less radiogold and expected to be less in cell injury had also lost their lymphocytes, but showed a marked proliferation of plasma cells in the medullary cord and large basophilic cells in the area of lymph follicles. The data suggest that the impaired immune response will be due to the failure of the macrophages in releasing the informational substance for plasma cell specialization and for antibody formation on account of possible inability in metabolizing the ingested antigen by the injured macrophages.

*PMID: 4239068 [PubMed - indexed for MEDLINE]

Acta Med. okayama 22, 113—122 (1968)

SUPPRESSION OF ANTIBODY FORMATION BY THE RES-BLOCKADE

II EFFECTS OF THE RES-BLOCKADE WITH RADIOACTIVE GOLD COLLOID

Shinji OHBUCHI

Department of Pathology, Okayama University Medical School, Okayama
(Director: Prof. S. Seno)

Received for publiction, April 24, 1968

In the previous paper (1) the author presented the fact that the blockade of the RES by PVP (polyvinylpyrrolidone) resulted in the suppressed proliferation of lymphoid cells as well as erythroid cells, suggesting that some information for the specialization of these cells should be transmitted from the RES cells and this would be easily arrested by the blockade of the RES by PVP.

On the other hand, the release of the informational substance for antibody formation from macrophage will not so severely be arrested, as the proliferated plasma cells proved to have a well retained potency for the antibody formation, though PVP could induce some delay in antibody formation comparing to the control.

OSOGOE (2) reported recently that there should be two phases for the antibody formation, as revealed by treating the animals with each component of adjuvant and antigen; the first phase is the proliferation of the cells having no specific activity and the second phase is the specialization of these proliferated cells so as to synthesize the specific protein, the antibody. Therefore, it will reasonably be deduced that for the specialization to synthesize antibody or specific somatic protein the cells may be conducted by the information from that for the cell specialization itself as immunologically competent cells.

The result seems to be consistent with the facts that the suppressive effect of irradiation on the antibody formation can be seen only in the initial stage (3, 4, 5, 6), but no suppressive effect in the stage of established antibody response (7, 8, 9, 10).

In the former experiment (1) the plasma cells proliferated by antigenic stimulation even in the area where the RES had been heavily loaded with PVP suggesting no direct communication between the function of the RES and the plasma cell proliferation, but there is a possibility that

the blockade of the RES with PVP will not be so harmful as to intercept the information for the plasma cell differentiation. To make clear this point the author intended to observe the plasma cell proliferation and antibody formation in the environment where the macrophages were severely damaged by ingesting radioactive gold colloid, as the plasma cells are known to be highly radioresistant (11) and yet the X-irradiation in high dose results in inhibition of antibody formation (3, 8, 9, 10), and yet it has also been reported that the RES cells, their morphological structure and phagocytic activity, are highly radioresistant (12, 13, 14) but the metabolizing ability of the ingested substances is very sensitive to irradiation (15, 16).

The present paper deals with the immunological and histological observations of the rats receiving the repeated injections of radiogold followed by the challenge of bovine serum albumin (BSA).

MATERIALS AND METHODS

Fifty young adult male rats, weighing 250–300 g were used; thirty animals for the study of the RES blockade by the intravenous injection of radiogold and the other twenty animals for the study of the effect of antigenic stimulation after the intravenous injection of radiogold.

Thirty animals to be used for the observation of the RES blockade by radiogold were divided into two groups, 10 and 20 animals respectively. The ten animals served as control and the other twenty animals were used for the clearance test. They were further divided into 4 groups, 5 animals each. The first group received the single injection of 5 mc of radiogold intravenously and one week later the clearance test was made. The second group received two injections of radiogold once a week for 2 weeks, the third group three injections for 3 weeks and fourth group four injections for 4 weeks. And in each group one week after the last injection of radiogold the clearance test was performed by injecting radioactive iron colloid intravenously, 2 μc per animal.

The results were compared with those from 10 controls receiving no pretreatment with radiogold and injected with iron colloid.

Twenty animals to be used for the study of the effect of the RES blockade on antibody formation were divided into two groups, 10 animals each. The first group received radiogold intravenously, 5 mc once a week for 5 consecutive weeks, and 25 mc as total.

The animals in other group served as the control and received the intravenous injection of saline solution. Ten animals, 5 animals each from these two groups, were challenged by the antigenic stimulation with BSA injection 24 hours after the administration of radiogold or saline. In other 10 animals each from 2 groups, blood cell count, hemoglobin level and hematocrit value were observed.

After 5 weeks observation all the animals were secrificed by decapitation.

Twenty-four hours before secrifice all the animals received the intraperitoneal injection of an excess amount of iron colloid for the purpose to examine the phagocytic activity of the RES in histological section.

The radioactive gold colloid used as the blockading agent was obtained from Dainabbot Laboratories, 5 mc/ml (1).

For the clearance test radioactive iron (chondroitin sulfric acid iron, Fe-CSA) was used, 5 μc ^{59}Fe in 1 ml solution. This radioactive iron was donated by the courtesy of Dainihon Pharmaceutical Inc., Osaka. In each observation 2 μc of radioactive iron was injected into penis vein and blood samples were taken and their radioactivity was estimated just as the previous experiment (1).

As the antigen Armour's crystalline bovine serum albumin was used.

The antigenic stimulation was performed with the same dose and same intervals as the former experiment. Antibody titration was evaluated by two-fold serial dilution technique by sheep red cell hemagglutination reaction (17).

RESULTS

The intravenous administration of radiogold resulted in a marked hemolysis in all the experimental animals. Red cell count decreased moderately during the first week and anemia became marked as the frequency of radiogold injection increased. Hemoglobin level did not show any significant change during the first week but later lowered gradually in proportion to the decrease in red cell count (Fig. 1).

The anemia thus developed proved to be refractory to the administration of iron. White blood cell count showed a striking decrease with a rapid fall of both granulocyte and lymphocyte numbers in the circulating blood during the first week but thereafter the decreasing-rate slowed down (Fig. 2).

The clearance test made by introducing colloidal radioactive iron, 2 μc per animal, revealed an impaired clearance rate by the single injection of radiogold. But repeated injections of radiogold did not show any significant effect in aggravating the RES function (Fig. 3).

The animals which received antigenic challenge 24 hours after the radiogold injection showed an extremely low potency of the antibody formation. The additional booster injection did not bring the level of serum antibody to that of the control even after the eighth day or more (Fig. 4).

Histological observation of the animals which received the injection of radiogold revealed significant changes in spleen, liver, lymph nodes and bone marrow in their cellular configuration.

The intravenously administered radiogold particles were found being trapped by the RES cells of these organs, most markedly in Kupffer cells

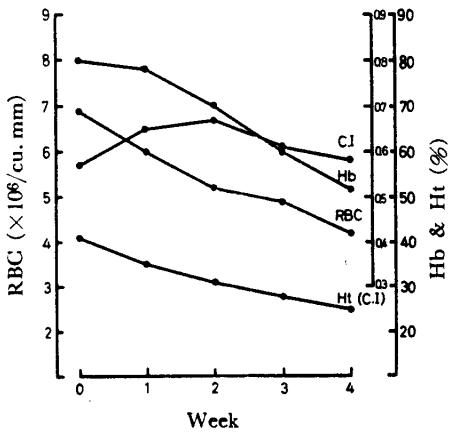


Fig. 1. Changes in red blood cell number, hemoglobin level and hematocrit value of the rats induced by the repeated intravenous injections of radiogold, 5 mc once a week for 5 consecutive weeks, 25 mc as total.

Each curve shows the mean value of 5 animals. RBC; red blood cell, Hb; hemoglobin level, Ht; hematocrit value, CI; color index.

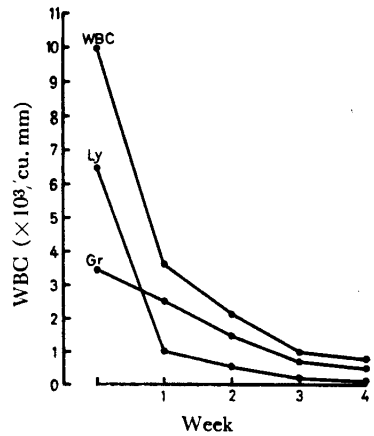


Fig. 2. Changes in white blood cell count of the rats. The value are of the same animals appearing in Fig. 1 and each curve gives the mean value of 5 animals. WBC; white blood cell, Ly; lymphocyte, Gr; granulocyte.

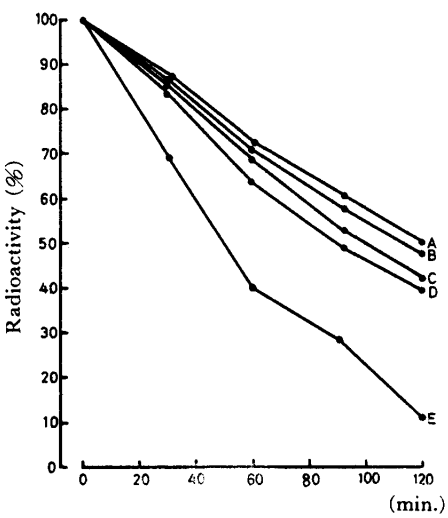


Fig. 3. Clearance rate in removal of radioactive iron in various preliminary doses and periods of intravenous injections of 5 mc of radiogold per week. Each curve gives the mean value of 5 animals. A; 4 injections of radiogold for 4 weeks, 20 mc as total.

B: 3 injections of radiogold for 3 weeks, 15 mc as total.

C: 2 injections of radiogold for 2 weeks, 10 mc as total.

D: one injection of radiogold for a week.

E: clearance rate obtained from normal rats.

of the liver, moderately in sinus-lining and reticular cells in spleen and somewhat slightly in the lymph nodes and bone marrow. A striking histological changes to be noted is a very active phagocytosis of the RES cells for erythrocyte in spleen and lymph nodes, especially, red pulp of

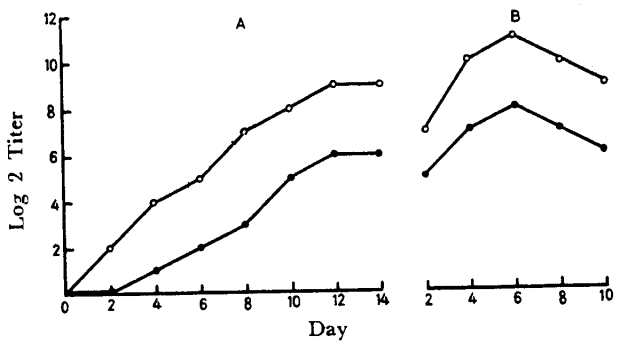


Fig. 4. Serum antibody titration against BSA, giving mean value of 5 animals
A: initial antigenic challenge with 0.5 ml of 1 % BSA with Freund's complete adjuvant twice with one week interval
B: booster injection of 0.5 ml of 1 % BSA intravenously 2 weeks after the last initial antigenic challenge
○—○ : control
●—● : antibody titration of animals treated with radiogold

spleen and medullary sinusoid of lymph nodes, though spleen was atrophic with the disappearance of lymph follicles, and reduced in cellularity being accompanied by the generalized fibrosis. Large basophilic cells were found in the area surrounding the collapsed lymph follicles and central arteries.

No other significant changes were found in the liver parenchymal cells, but bone marrow was rather poor in its cell component; the granulopoiesis was severely arrested and the picture of erythroblastic islet appeared distinct, having reticular cell in the center which have contained gold colloid. The thymus was severely atrophied and replaced by fatty tissue.

The antigenic challenge of the animals pretreated with radiogold injection showed no reactive proliferation of lymphocytes and even of plasma cells in red pulp of spleen, which was of the general appearance as in the control receiving antigenic challenge without radiogold pretreatment.

Nearly complete disappearance of lymph follicles in lymph nodes induced by radiogold did not showed any recovery tendency by the antigenic challenge but showed a marked proliferation of plasma cells.

Histological observation of iron colloid uptake by the RES of these animals pretreated with radiogold revealed a well-retained phagocytic activity indicating that the ingestion of radiogold by the RES did not lower their phagocytic activity.

DISCUSSION

As demonstrated in this experiment, the radioactive gold colloid particles injected intravenously were selectively trapped by the RES, Kupffer cells of the liver, sinus-lining and reticular cells in spleen and lymph nodes, and reticular cells in bone marrow, with a marked atrophy of lymph follicles in spleen and lymph nodes and suppressed granulopoiesis in bone marrow.

Erythropoiesis was not severely arrested but the red cell membrane should be altered, as they were readily hemolyzed and phagocytized by the RES. Reflecting these histological and cellular changes, lymphopenia and granulocytopenia with moderate anemia have been observed in the circulating blood. The RES cells of liver and spleen were heavily loaded with the radiogold particles from which an impaired clearance rate resulted, though the RES cells appeared not to be damaged morphologically.

The moderately impaired clearance rate observed in the present experiment seems to conflict with the data given by many investigators who observed the unchanged clearance rate in the animals exposed to lethal total body irradiation (18, 19, 20, 21) but this difference indicates that the macrophages ingested radiogold were injured more selectively and severely than those exposed to whole body irradiation.

However, the phagocytic activity of the RES was not completely suppressed even after the ingestion of a fairly large amount of radiogold, just as in the case of the RES in liver and spleen.

Therefore, it is very difficult to destroy the macrophages completely by giving radioactive metals. But lymphocytes, because of the high radiosensitivity, should have disappeared by the effect of the radiogold trapped by the RES.

The antigenic challenge to these animals revealed a markedly lowered potency in antibody formation. The atrophied lymph follicles did not become hypertrophic both in spleen and lymph nodes even 10 days after the booster. But plasma cells appeared in the medullary cord of lymph nodes and probably this plasma-cytic reaction acted as the source of antibody formation.

The reduced antibody titration observed in the present experiment is closely correlated to the decreased number of plasma cells which will be reasonable to the formation of circulating antibody against BSA.

Plasma cells appeared in some lymph nodes but not in spleen.

The arrested plasma cell proliferation in spleen will not be due to

the direct effect of irradiation by radiogold, because it is well known that plasma cell is redioresistant as it has been proved that the established antibody response is not diminished by irradiation (7, 8, 9, 10) and the ability of plasma cell to survive irradiation explain the radioresistance of established antibody response (11).

Generally, the suppression of antibody formation by irradiation can only be induced when the animals are irradiated in the initial phase of immune response (3, 4, 5, 6) and the irradiation only, a few hours after the antigenic challenge, shows no suppressive effect on the antibody production (3, 22).

This fact suggested that x-irradiation arrested the information from macrophages to plasma cell, but never destroyed the plasma cell themselves.

According to FISHMAN (23, 24) the informational substance may be m-RNA, but more recent work by ASKONAS (25) suggested that the RNA-antigen complex may be essential, which will be formed by degrading the ingested antigen.

Another work by DONALDSON and others (15, 16) indicates that by x-irradiation the macrophages may ratain the phagocytic activity but lose ability of ingesting or degrading antigen. These evidences clearly indicate that the macrophages damaged by intracellular irradiation by radiogold may keep their phagocytic activity but lose their antigen-degrading ability, resulting in the inability to release informational substance for the plasma cell proliferation by which ultimately specific antibody is produced.

SUMMARY

For the purpose of revealing the interaction between macrophages and plasma cells in relation to antibody formation and information for cell specialization, the proliferation of plasma cell by antigenic stimulation was observed in the rats whose RES had been previously injured by radiogold.

The production of the circulating antibody was markedly suppressed by the pretreatment with radiogold.

Histological observation revealed that the plasma cells and lymphocytes were completely obliterated and the tissues were replaced by the basophilic cells and fibroblastic cells.

Lymph nodes which contained less radiogold and expected to be less in cell injury had also lost their lymphocytes, but showed a marked proliferation of plasma cells in the medullary cord and large basophilic cells

in the area of lymph follicles.

The data suggest that the impaired immune response will be due to the failure of the macrophages in releasing the informational substance for plasma cell specialization and for antibody formation on account of possible inability in metabolizing the ingested antigen by the injured macrophages.

REFERENCES

1. OHBUCHI, S.: *Acta Med. Okayama* 22 (in press)
2. OSOGOE, B.: *Acta Path. Jap.* 17, 267, 1967
3. DIXON, F.J., TALMAGE, D.W. and MAURER, P.H.: *J. Immunol.* 68, 693, 1952
4. TALIAFERRO, W.H., TALIAFERRO, L.G. and JAROSLOW, B.N.: Radiation and the Immune Response 31, Academic Press, New York, 1964
5. DIXON, F.J. and MCCONAHEY, P.J.: *J. Expt. Med.* 117, 833, 1963
6. PRIBNOW, J.F. and SIVERMAN, M.S.: *J. Immunol.* 98, 225, 1967
7. SIMIC, M.M.: *J. Cell. Physiol.* 67, 129, 1967
8. TALIAFERRO, W.H.: *Ann. N.Y. Acad. Sci.* 69, 745, 1957
9. GARVER, R.M., SANTOS, G.W. and COLE, L.J.: *J. Immunol.* 83, 57, 1959
10. DAVIES, A.J.S., DOE, B., CROSS, A.M. and ELLIOTT, E.V.: *Nature* 203, 1039, 1964
11. MILLER, J.J. and COLE, L.J.: *J. Immunol.* 98, 982, 1967
12. TULLIS, J.L.: *Arch. Path.* 48, 171, 1949
13. KORNFELD, L. and GREEMAN, V.: *Radiat. Res.* 29, 433, 1966
14. KORNFELD, L. and GREEMAN, V.: *Radiat. Res.* 31, 889, 1967
15. DONALDSON, D., MARCUS, S., GYI, K.K. and PERKINS, E.M.: *J. Immunol.* 76, 192, 1956
16. NELSON, E.L. and BECKER, J.R.: *J. Infect. Dis.* 104, 13, 1959
17. STAVITSKY, A.B.: *J. Immuuol.* 72, 360, 1954
18. BARROW, J., TULLIS, J.L. and CHAMBER, F.: *Amer. J. Physiol.* 164, 822, 1951
19. WISH, L., FURTH, J., SHEPPARD, C. and STOREY, R.H.: *Amer. J. Roentgenol.* 67, 628, 1952
20. DI LUZIO, N.R.: *Amer. J. Physiol.* 181, 595, 1955
21. BENACERRAF, B., KIRVY-ROSENBERG, E., SEBESTYEN, M.M. and ZWEIFACH B.W.: *J. Exptl. Med.* 110, 49, 1959
22. TALIAFERRO, W.H., and TALIAFERRO, L.G.: *J. Infect. Dis.* 95, 134, 1954
23. FISHMAN, M.: *J. Expt. Med.* 114, 837, 1961
24. FISHMAN, M. and ADLER, F.L.: *J. Expt. Med.* 117, 595, 1963
25. ASKONAS, B.A. and RHODES, J.M.: *Nature* 205, 470, 1965

EXPLANATIONS

Photo. 1. Picture of the liver of the rat receiving the repeated injections of radiogold, 25 mc as total. Kupffer cells digesting a large amount of radiogold particles and unaffected parenchymal cells.

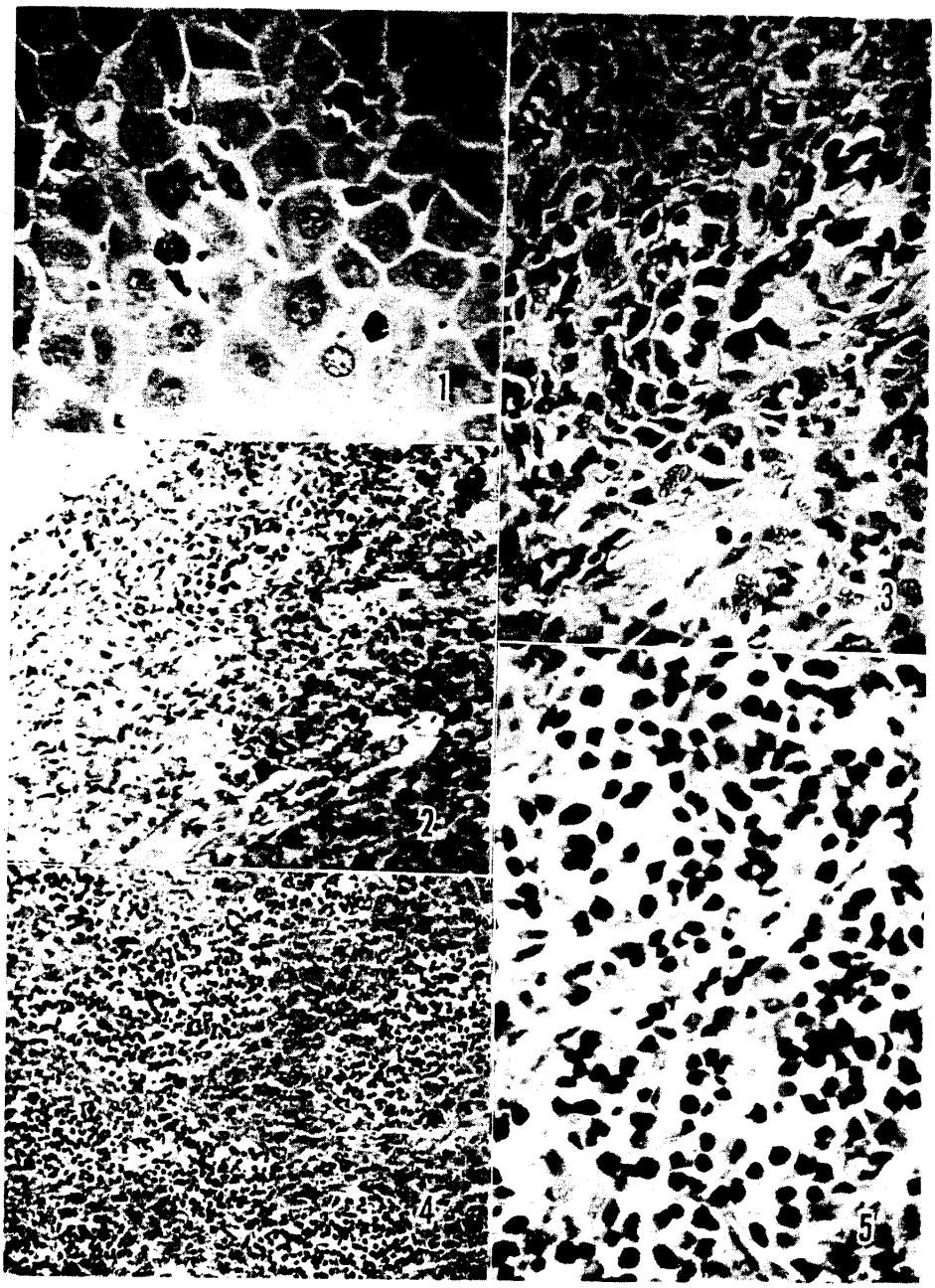
Photo. 2. Picture of the spleen of the rat receiving repeated injections of radiogold and then challenged with BSA. Large basophilic cells in the vicinity of central artery and markedly reduced cellularity accompanied by generalyzed fibrosis.

Photo. 3. Picture of the spleen of the same animal as Photo. 2. Large basophilic cells

surrounding central artery and complete disappearance of lymphocytes and plasma cells in red pulp.

Photo. 4. Picture of the mesenterial lymph node of the same animal as Photos. 2, 3. Cord-like proliferation of plasma cell in medullary sinusoid and complete disappearance of lymphocyte with faded lymph follicles in cortex.

Photo. 5. Picture of the mesenterial lymph node of the same animal as Photo. 4. Plasma cell proliferation in medullary sinusoid.


1
2
3
4
5