Acta Medica Okayama

Volume 22, *Issue* 3 1968 *Article* 2

JUNE 1968

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Suppreion of antibody formation by the res-blockade. Ⅲ. Effects of the res-blockade with methyl palmitate*

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Abstract

The rats which received the repeated intra peritoneal or intravenous injections of methyl palmitate showed a marked depreed phagocytic activity of the RES as shown by the clearance test with radioactive iron as well as by histological observations and a significantly suppreed antibody formation against the challenge by BSA. Differing from the cases of the blockade of the RES made by PVP or radiogold, the injection of methyl palmitate did not result in any injurious effect on the lymph follicles of lymph nodes and spleen and the plasma cells proliferation as revealed by the histological observation. Histochemical observations of iron phagocytosis of the RES done by Perls stain revealed that methyl palmitate suppreed the phagocytic activity of the Kupffer cells of the liver dramatically and also suppreed the phagocytic activity of the sinus-lining cells in spleen to a leer degree. The result indicates that the injection of methyl palmitate attacks the phagocytic function of the RES selectively and induces the reduced immune response of the organism without giving any damages to the proliferation of immunologically com petent cells. The fact suggests that the RES lowered in their phagocytic activity fails to produce the informational substance for immune response, showing a lower level in the antibody formation even in the presence of antigen and proliferating immunologically competent cells.

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*PMID: 4239072 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **22**, 137—146 (1968)

SUPPRESSION OF ANTIBODY FORMATION BY THE RES-BLOCKADE III EFFECTS OF THE RES-BLOCKADE WITH METHYL PALMITATE

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Received for publication, Fed. 10, 1968

In the previous papers (1, 2) the author indicated that the damage of the RES exposed to PVP (polyvinyl pyrrolidone) or radiogold resulted in the delay of the antibody formation or the lowered level of antibody production, probably due to the suppression of the production of the informational substance toward immunologically competent cells for their antibody formation.

Besides these, the data also suggested that the RES will have some function for the specialization of lymphoblast to lymphocytes and erythroblast to erythrocytes, as the specialization of these cells have severely been arrested by the blockade of the RES with PVP and radiogold.

However, inspite of a very severe damage of the RES induced by loading an amount of PVP or radiogold the complete inhibition of the immune response has never been attained.

This paper deals with the observation of the effect of methyl palmitate on the function of the RES as intermediator in the antibody response. Differing from the case of the RES-blockade by PVP or radiogold, the intravenous injection of methyl palmitate can suppress the antibody response without inducing the cell damage of immunologically competent cells in the lymphoid tissue and the lymphocyte level in circulating blood.

MATERIALS AND METHODS

Sixty Wistar strain young adult male rats, weighing 250 to 300 g were used : Forty animals for the clearance test and remaining 20 for antigenic challenge.

Forty animals used for the clearance test were divided into 2 groups, 20 animals each ; 20 for the observation of the effects of methyl palmitate on the phagocytic activity of the RES for radioactive iron colloid and the other 20 served as

control being treated with Tween 20, the solvent used for methyl palmitate. Twenty animals belonging to the first group received single intravenous injection of methyl palmitate, 250 mg, and thereafter divided into 4 subgroups, 5 animals each. Of these 4 groups the clearance test was performed at 6, 12, 24 and 48 hours after the administration of methyl palmitate by using radioactive iron colloid (^{59}Fe) just as in the cases reported in the previous papers (1, 2). The remaining 20 served as control and received a single injection of the solvent, Tween 20, and thereafter divided into 4 groups, 5 animals each and the clearance test was performed at 6, 12, 24 and 48 hours after the administration of the solvent.

The 20 animals for the observation of the antibody formation were divided into 2 groups, 10 animals each. The animals in the first group received 250 mg of methyl palmitate in 2 ml of 5 % glucose containing Tween 20 (0.1 %) from intraperitoneal or intravenous route daily for 14 consecutive days.

Intravenous administration of methyl palmitate was given once every 3 days throughout the experimental period because of the high mortality from daily intravenous injection. The ten animals in the second group served as control and received the injection of only 2 ml of solvent, Tween 20, through the same route at the same frequency as those in first group. The animals from these 2 groups, 5 animals each, were challenged by antigenic stimulation starting from 24 hours after the initial injection of methyl palmitate or Tween 20, and the other 5 animals of each group served as control and the hematologic observation was made on these animals.

Twenty-four hours before secrifice all the animals received an excess amount of colloidal iron (Blutal, Dainihon Pharmaceutical Inc., Osaka) intraperitoneally or intravenously for the purpose of detecting the phagocytic activity of the RES with histochemical reaction.

Besides these, liver, spleen, mesenterial lymph nodes and bone marrow were fixed in 10 % formol, and paraffin sections were made for routine histological observation by hematoxyline-eosin stain.

Methyl palmitate used was the product of Nakarai Chemicals, Kyoto.

Before the use 250 mg of the agent were added to 2 ml of 5 % glucose containing Tween 20 (0.1%), heated to 70°C and emulsified by using injection syringe and was administered to a animal while warm (35—40°C).

For the clearance test radioactive chondroitin sulfric acid iron (^{59}Fe-CSA), containing 5 μc of ^{59}Fe, 4 mg of iron and 20 mg of chondroitin sulfric acid, in 1 ml of solution, Dainihon Pharmaceutical Inc., Osaka, was used.

Two μc of radioactive iron (^{59}Fe-CSA) was injected into penis vein and blood samples (0.1 ml) were taken in heparinized hematocrit tube at certain intervals from retro-orbital sinus, and radioactivity was measured by the well-type scintillation counter and clearance curves were drawn.

As the antigen Armour's crystalline BSA was used. 0.5 ml of 1 % BSA in physiological saline and 0.5 ml of Freund's complete adjuvant (Difco) conjugate was injected into foot pads, subcutaneous and intramuscular tissues, twice at one week interval.

Antibody titration was evaluated by two-fold serial dilution technique

employing BOYDEN's sheep red cell hemagglutination reaction (3).

For the observation of phagocytic activity of peritoneal macrophages *in vitro* mouse Ehrlich ascites was used by modifying the method of YOKOMURA and others (4), peritoneal macrophages were obtained from Ehrlich tumor ascites previously treated with methyl palmitate or Tween 20.

The animals 5—7 days after the tumor cell inoculation received the intraperitoneal injection of methyl palmitate emulsion containing 50 mg of methyl palmitate and one hour later the ascites macrophages were obtained and they were incubated for 10 and 20 minutes at 37°C with Fe-CSA and the phagocytosis was observed as in the previous experiments (1, 2).

In addition, phagocytic activity of the mesenterial cells of normal mouse for Fe-CSA colloid was also observed 24 hours after the single intraperitoneal injection of 50 mg of methyl palmitate emulsion, iron colloid was injected, and the animals were sacrificed 2, 6, 12 and 24 hours after the iron injection. The mesentery was extended on an object glass, fixed with methanol and observed by Perls-Stieda stain.

RESULTS

The repeated intraperitoneal and intravenous injections of methyl palmitate, in a vast amount did not induce any significant changes in the cellular components of the circulating blood. An active hematopoiesis in bone marrow and well-developed lymph follicles in spleen and lymph nodes were retained as revealed by histological observation. However, a marked reduction in clearance rate in removal of iron colloid from the circulating blood was observed 12, 24 and 48 hours after the single intravenous injection of methyl palmitate, though 6 hours later the clearance rate of iron colloid was maintained within normal ranges (Fig 1). All the animals used for the clearance test were killed 24 hours after the intravenous injection of Fe-CSA.

Histological observation revealed that the injected iron colloid particles were accumulated chiefly in Kupffer cells of the liver and sinus-lining and reticular cells in spleen and some reticular cells of bone marrow and lymph nodes.

In the animals receiving pretreatment with methyl palmitate iron colloid was accumulated much less in Kupffer cells in the liver comparing to that of the control pretreated with Tween 20 alone.

Kupffer cells in the liver of the animals treated with methyl palmitate also showed a phagocytic activity but they were not enlarged or swollen as the RES cells of the control animals loaded with iron colloid.

On the other hand, a marked phagocytosis of iron colloid by sinus-lining and reticular cells of the red pulp, espescially those lying in the

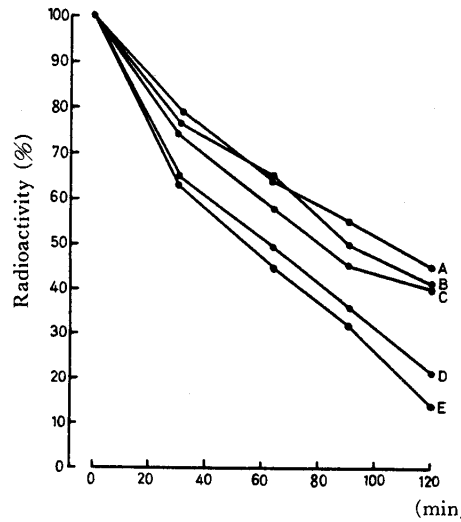

Fig. 1. Clearance rate in removal of radioactive iron at various intervals after the single intravenous injection of 250 mg of methyl palmitate. Each curve gives mean value of 5 animals.
A : 48 hours after the single injection of methyl palmitate.
B : 24 hours after the single injection of methyl palmitate.
C : 12 hours after the single injection of methyl palmitate.
D : 6 hours after the single injection of methyl palmitate.
E : clearance rate obtained from the control receiving the solvent, Tween 20.

vicinity of the marginal zone of lymph follicles were observed in the animals treated with methyl palmitate as well as in control treated with Tween 20. All these cells were more or less swollen accumulating iron colloid extremely. There was observed also little difference in iron colloid accumulation of the RES of lymph nodes and bone marrow between those untreated and treated with methyl palmitate.

Three of the 5 animals pretreated with the repeated administrations of methyl palmitate proved to be extremely poor in antibody formation as revealed by the test 14 days after the initial antigenic challenge by BSA comparing to those pretreated with Tween 20. But the remaining 2 animals showed the antibody formation nearly the same as that of control (Fig. 2). All three animals showed the poor antibody titration also showed a markedly reduced clearance rate in removal of iron colloid introduced into blood stream.

The peritoneal macrophages from Ehrlich ascites tumor of mice taken one hour after the single intravenous injection of 50 mg of methyl palmitate in emulsion contained a few oil droplets in their cytoplasm but

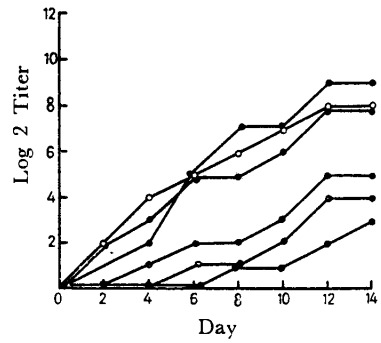

Fig. 2. Serum antibody titration against BSA. ○—○ ; antibody titration obtained from the control receiving Tween 20, giving mean value of 5 animals. •—• ; antibody titration of animal treated with methyl palmitate.

showed no significant morphologic changes. The phagocytic activity of these cells tested by incubating with iron colloid for 10 and 20 minutes at 37°C proved to be extremely low or completely lost (Table. 1).

Table 1 Phagocytic activity of the peritoneal macrophages obtained from Ehrlich ascites pretreated with *25 mg and **50 mg of methyl palmitate in incubating with iron colloid for 10 and 20 minutes.

Peritoneal Macrophages \ Incubation Time (min.)	10	20
* Methyl Palmitate Treated Peritoneal Macrophages	+	+
** Methyl Palmitate Treated Peritoneal Macrophages	−	−
Control	+	+

However, the cells from the animals receiving 25 mg of methyl palmitate showed a marked phagocytic activity as well as the cells from the control animals.

Mesenterial macrophages from the animals pretreated with methyl palmitate, 50 mg once intraperitoneally, showed a marked reduction in their phagocytic activity for iron colloid. Iron was introduced intraperitoneally 24 hours after the intraperitoneal injection of methyl palmitate and the observation was made at varying time intervals but nearly the same results were obtained in those taken 2, 6, 12 and 24 hours after the intraperitoneal iron injection.

Histological observations of various organs of the rats made after the repeated intraperitoneal and intravenous injections of methyl palmitate did not show any significant changes excepting some in lungs of lipid embolism and related changes. The picture to be noticed is that the RES cells of liver, spleen and lymph nodes showed no swelling nor proliferation different from the cases exposed to general RES-blockading agents.

The atrophic changes of lymph follicles of spleen and lymph nodes, which are also generally induced by injecting other RES-blockading agents, were not at all observed. The cellular configuration of spleen and lymph nodes appeared unchanged.

Antigenic challenge was much less effective in the induction of the proliferation of reticular cells and immunologically competent cells.

The phagocytic activity test of the RES revealed no iron particles in Kupffer cells in the liver after the intraperitoneal injection of iron colloid, and much less in sinus-lining cells of spleen, while the phagocytic cells in lymph nodes, bone marrow and lung showed intact phagocytic activity, accumulating a large amount of colloidal iron.

The intravenous injection of iron colloid gave somewhat different picture. Kupffer cells in the liver of the animals treated with methyl palmitate phagocytized some iron colloid, though they showed no swelling by ingesting iron colloid and the RES cells in spleen exhibited rather active phagocytosis as compared with that of Kupffer cells of the liver.

DISCUSSION

As demonstrated in this experiment, repeated intraperitoneal and intravenous injections of methyl palmitate have resulted in the heavily suppressed phagocytic activity of Kupffer cells of the liver and the sinus-lining cells of spleen and the depressed antibody formation without any damages to lymphoid and hematopoietic tissues.

The selective depression in the phagocytic activity of the Kupffer cells of the liver and sinus-lining cells in spleen seems to indicate that methyl palmitate introduced into the circulating blood as gross emulsion particles is incapable of permeating capillary endothelial barrier and only those situated inside the capillary endothelium and directly exposed to methyl palmitate can be selectively affected. Because the emulsion is very unstable and the particles grow bigger even during a short time of injection period.

Thus the organ specific suppressive effect of methyl palmitate on phagocytic activity is possibly not due to any specific tissue affinity of

methyl palmitate but due to the large size of emulsion particles.

It is well known that generally a single lipid complex, e. g. glyceril trioleate, enhances the antibody response by stimulating the RES (6, 7, 8) but some lipid acts as to depress markedly the functional activity of the RES (9, 10, 11, 12). These facts seem to suggest that a variety of lipids might act as the controller of the phagocytic activity of the RES.

Concerning the biological action and chemical structure, some investigators(12) have suggested the alcoholic component might be more essential than fatty acid residue in determining the biological characteristic of the ester, e. g. glycerol ester stimulates the phagocytic activity of the RES, while ethyl ester suppresses the activity of the RES.

But other investigators (8) proposed that the dyspeptic or poorly digesting phagocytic cells may be a reflection of the presence of the lipid within the cells, which might adversely influence their enzymic activity. As the phagocytic activity is dependent on the energy by anaerobic glycolysis, the mechanism may be of energy suppression.

Another reasonable explanation of the mechanism may come from the difference in melting point, i. e. saturated lipid such as methyl palmitate as well as ethyl stearate will be incorporated into the cell membrane making it rather rigid while unsatulated fatty acids may act as to enhance the phagocytic actitvity because of their low melting point by making the cell membrane flexible ; the rigid cytomembrane may be difficult for engulfing, whereas the flexible membrane enables the formation of invagination for engulfing.

At present there is no conclusive evidence to decide which mechanism is responsible for the depressing effect of methyl palmitate on the phagocytic activity of the RES but the important thing is that the suppression of immune response can be obtained by suppressing the phagocytic activity of the RES without giving any damaging effect on the proliferation of immunologically competent cells.

Thus, the present experiment clearly indicates that the suppressed phagocytic activity of antigen by the RES results in a poor production of informational substance to the immunologically competent cells.

Concerning the informational substance itself, some complex of antigen and its decomposed substance combined with RNA are found in the lysosomal fraction of Kupffer cells of the liver (13) and such antigen-RNA complex is supposed to be informational substance (14).

If the phagocytosis of an antigen and the subsequent solubilization or digestion of the antigenic material is inhibited or markedly reduced by the RES depression, little degraded antigenic material would be made avail-

able for antibody forming cells and the release of the informational substance for antibody formation would be correspondingly reduced.

The fact that the animals having the RES affected by methyl palmitate maintain the circulating antigen at a high level indicates that the phagocytosis of antigen by the RES is suppressed equally just as in the case of phagocytosis of colloidal metal particles.

It has been demonstrated (15) that the circulating antibody appears after the level of circulating antigen has decreased.

Therefore, it is concluded that most of the antigens introduced are trapped by the RES or macrophages, and some informational substance is released to the antibody forming cells.

The methyl palmitate causes the reduction in the phagocytic activity and probably the reduced production of the informational substance to the immunologically competent cells, and consequently, antibody formation will be correspondingly reduced, even though the lymphocytes and plasma cells would have proliferated.

SUMMARY

The rats which received the repeated intraperitoneal or intravenous injections of methyl palmitate showed a marked depressed phagocytic activity of the RES as shown by the clearance test with radioactive iron as well as by histological observations and a significantly suppressed antibody formation against the challenge by BSA.

Differing from the cases of the blockade of the RES made by PVP or radiogold, the injection of methyl palmitate did not result in any injurious effect on the lymph follicles of lymph nodes and spleen and the plasma cells proliferation as revealed by the histological observation.

Histochemical observations of iron phagocytosis of the RES done by Perls stain revealed that methyl palmitate suppressed the phagocytic activity of the Kupffer cells of the liver dramatically and also suppressed the phagocytic activity of the sinus-lining cells in spleen to a lesser degree. The result indicates that the injection of methyl palmitate attacks the phagocytic function of the RES selectively and induces the reduced immune response of the organism without giving any damages to the proliferation of immunologically competent cells.

The fact suggests that the RES lowered in their phagocytic activity fails to produce the informational substance for immune response, showing a lower level in the antibody formation even in the presence of antigen and proliferating immunologically competent cells.

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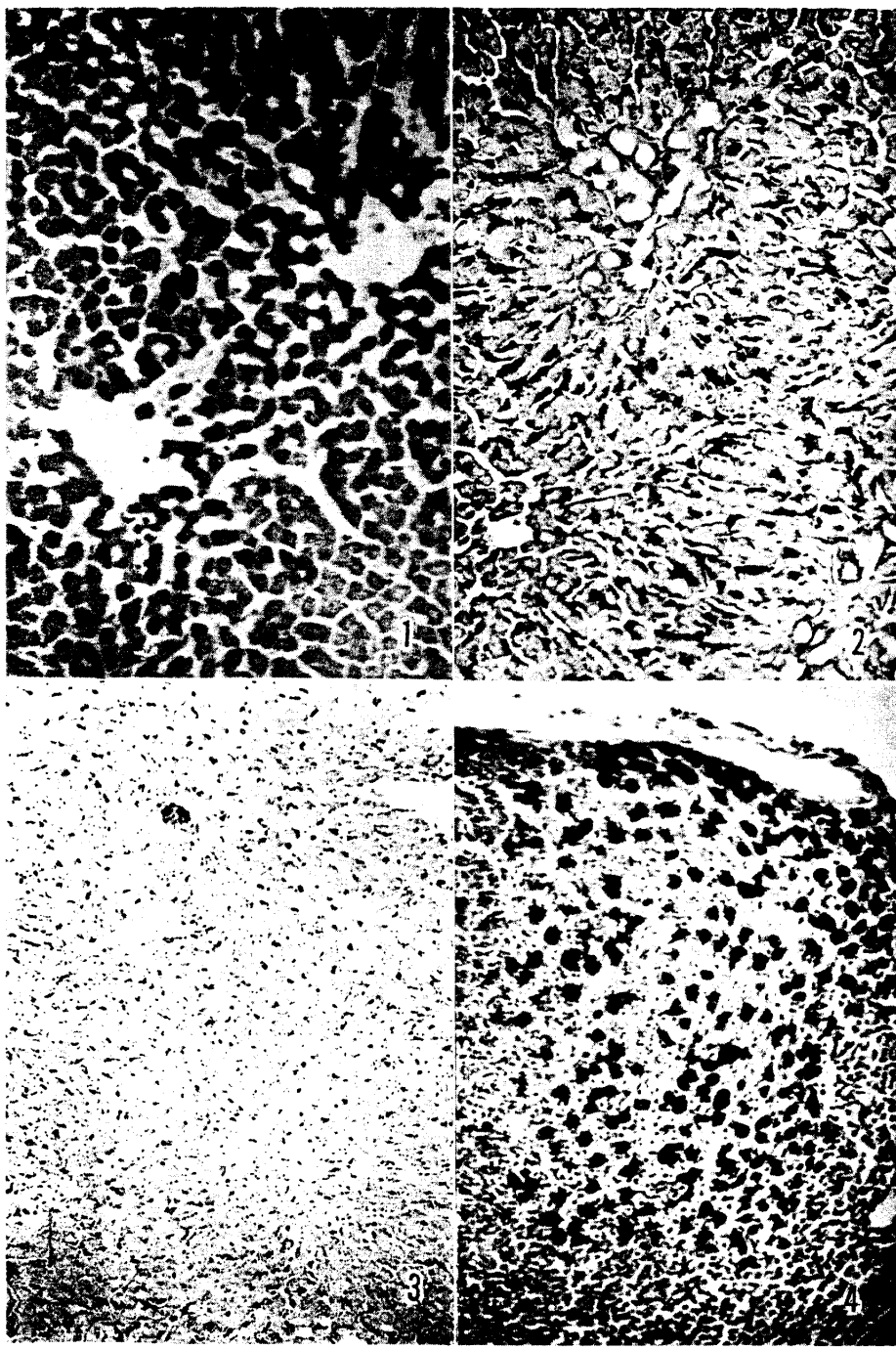
Photo. 1 Picture of the liver of the rat after intraperitoneal administration of colloidal iron, pretreated with repeated intravenous or intraperitoneal injections of methyl palmitate, 250mg per day daily for 14 days. Lack of the introduced iron particles in Kupffer cells of the liver.

Photo. 2 Picture of the liver of the control after intraperitoneal injection of colloidal iron, pretreated with repeated intravenous or intraperitoneal injections of the solvent, Tween 20. Swelling and enlargement of Kupffer cells of the liver by digesting a large amount of colloidal iron.

Photo. 3 Picture of the liver of the rat after intravenous injection of colloidal iron, pretreated with repeated intravenous or intraperitoneal injections of methyl palmitate. Markedly reduced phagocytic activity for colloidal iron and lack of enlargement or swelling of Kupffer cells of the liver.

Photo. 4 Picture of the mesenterial lymph node of the same animal as Photo. 1. Swollen and enlarged sinus-lining and reticular cells trapping a large amount of colloidal iron.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