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Antigen–Antibody Complexes in the Immune Response

I. ANALYSIS OF THE EFFECTIVENESS OF COMPLEXES ON THE PRIMARY ANTIBODY RESPONSE

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Summary. Soluble crystalline bacterial α-amylase (BαA)-mouse anti-BαA antibody complexes (Ag–Ab complexes) elicited a primary antibody response in mice with a single intravenous injection, while free BαA could not. The response was dose dependent. Ag–Ab complexes were not only phagocytosed but also degraded more rapidly than free BαA *in vivo* and *in vitro* but these characteristics themselves were not important for immunogencity of the complexes.

The Ag–Ab complexes phagocytosed by cells in normal spleen and lymph node elicited a primary antibody response when injected into non-irradiated mice but the response was suppressed when anti-BαA antibody was simultaneously injected. On the other hand, free BαA phagocytosed by cells could not elicit the response.

The degraded products of complexes phagocytosed by normal spleen and lymph node cells were highly immunogenic and probably retain antigenic fragments. They elicited an even higher primary antibody response than the original complexes and were also more effective in eliciting a secondary response from primed cells than the original complexes or free BαA. The degraded products of free BαA, however, were ineffective not only for the primary response but also for primed cells.

Ag–Ab complexes prepared with heterologous rabbit antibody were ineffective for the primary antibody response.

INTRODUCTION

Up to now, several investigators have reported that Ag–Ab complexes are more immunogenic than free antigen for the primary antibody response (Terres and Wolins, 1959; Segre and Kaeberle, 1962; Dennert, 1971), for priming (Uhr and Baumann, 1961) and for the secondary antibody response (Hamaoka and Kitagawa, 1971). However, the exact mechanisms are still obscure and this is probably because we still lack enough knowledge about the differences of responses to complexes and free antigen. Almost the only presently known difference, which seems to be related to the antibody response, is that the uptake of antigen by phagocytes is facilitated by the presence of antibody (Weigle, 1958; Sorkin and Boyden, 1959; Patterson, Suszko and Pruzansky, 1962).

Based on Fishman's studies (1961), macrophages, one of the representative cell population of phagocytes, are said to be important for the induction of antibody synthesis. Therefore, the superior immunogenicity of Ag–Ab complexes may be tentatively explained

by thinking that complexes are taken up by phagocytes and particularly by macrophages quantitatively more than free antigen and that this difference leads to the difference of immunogenicity. The present experiments were carried out to test whether this is indeed so.

MATERIALS AND METHODS

Animals

The ddO albino mice used in the present study were supplied from the Central Breeding Laboratory of Experimental Animals of Osaka University.

Antigen

The antigen used was crystalline bacterial α-amylase (BαA) derived from *Bacillus subtilis*, obtained from Nagase Sangyo Co., Ltd. Radioiodination of BαA was done by the chloramine-T method (Greenwood, Hunter and Glover, 1963) using ^{131}I to obtain a specific activity of 0·5–1 mCi/mg.

Antibody

Anti-BαA antisera obtained from mice and a rabbit immunized with BαA in Freund's incomplete adjuvant were used as sources of antibody. The antibody titres were determined by neutralization of the amylase activity of BαA, described by Hamaoka, Kitagawa, Matsuoka and Yamamura (1969).

Ag–Ab complexes

BαA-anti-BαA mouse or rabbit antibody complexes were prepared *in vitro* by incubating BαA with either antiserum containing equivalent, unless otherwise noted, units of antibody at 37° for 1 hour. Unless otherwise noted the word 'Ag–Ab complexes' means the complexes prepared with mouse antibody. Free BαA was incubated with normal mouse serum.

Antibody assay

Passive haemagglutination was used. BαA was conjugated to homologous ddO mouse red blood cells by $CrCl_3$ (Kishimoto, Tsuyuguchi and Yamamura, 1968).

Every test sample was heat inactivated at 56° for 30 minutes before titration. Antibody titres were expressed as the geometric mean of the reciprocal of the highest dilution of the positive tubes.

Preparation of normal spleen and lymph node cells and in vivo *and* in vitro *culture*

Spleens and mesenteric and cervical lymph nodes of normal mice were minced and passed through an 80-cm mesh stainless steel sieve and the resulting cell suspensions were washed well with Hanks's balanced salt solution (HBSS) and adjusted to the appropriate cell concentrations with Eagle's minimum essential medium (MEM). After incubation with complexes or free BαA *in vitro*, these cells were either cultured *in vitro* or transferred into the recipients with or without normal lymphoid cells. The recipients of the above *in vitro* treated cells with normal lymphoid cells had previously been exposed to total body X-irradiation of 600 r (25 mA; 250 KV; 1·0 mm Al filter, dose rate of 133 r/min at 58 cm from the target).

Preparation of primed cells and in vivo *culture*

Primed cells were obtained from the spleens and lymph nodes of the mice which had been immunized with BαA in Fruend's incomplete adjuvant. After appropriate treatment *in vitro*, these cells were transferred into 600 r X-irradiated recipients.

RESULTS

PRIMARY ANTIBODY RESPONSE ELICITED BY Ag–Ab COMPLEXES

The first experiment was carried out to test whether, in our experimental conditions, Ag–Ab complexes would elicit a primary antibody response. Normal mice were injected intravenously or intraperitoneally with either 20 μg complexes formed at equivalence or 20 μg free BαA and were bled and then sera titrated several times after treatment (Fig. 1). All

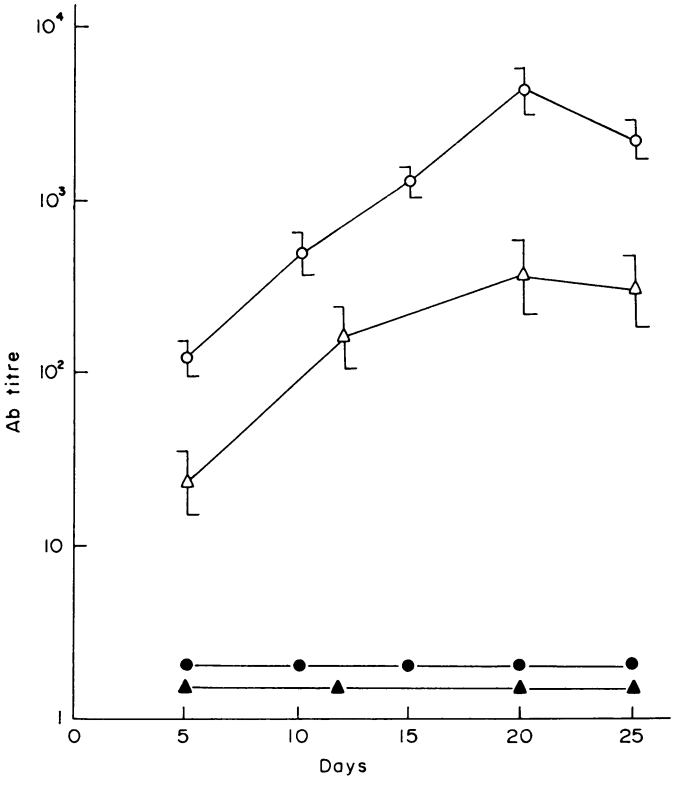

FIG. 1. Primary antibody response by Ag–Ab complexes. Normal mice were injected intravenously or intraperitoneally with 20μg complexes or 20μg free BαA. Open circles, complexes given i.v.; solid circles, free BαA given i.v.; open triangles, complexes given i.p; solid triangles, free BαA given i.p. Each symbol represents the mean value of eight to ten mice. Bars represent the standard errors of the logarithimically transformed data.

the recipients given complexes showed a clear primary antibody response but those given free BαA did not. The mice given complexes intravenously showed a much higher and less individually variable response than those given complexes intraperitoneally. Therefore, in every subsequent experiment only intravenous injections were made.

TABLE 1

EFFECT OF ANTIBODY DOSE ON PRIMARY ANTIBODY RESPONSE BY Ag–Ab COMPLEXES

Ag (μg)	Ab (units)	Ratio of antigen to antibody Ag : Ab	Antibody titre at 10th day
10	6·6	1 : 1/5	33
10	33	1 : 1	592
10	165	1 : 5	150

Eight to ten mice were used for the recipients of each group.

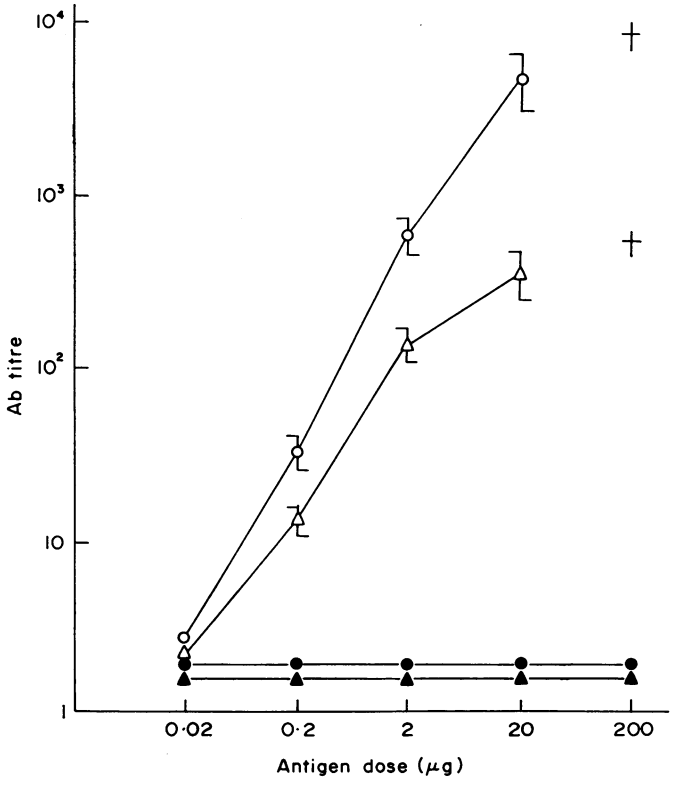

FIG. 2. Dose response curves of primary antibody response by Ag–Ab complexes. Normal mice were injected i.v. either with 0·02, 0·2, 2, 20 and 200 μg complexes or with the corresponding amounts of free BαA. They were bled 10 and 20 days later, respectively. Open circles, mice given complexes bled at 20th day; solid circles, mice given free BαA bled at 20th day; open triangles, mice given complexes bled at 10th day; solid triangles, mice given free BαA bled at 10th day. Each symbol represents the mean value of eight to ten mice. Crosses mean the death of all the recipients.

To test whether this antibody response was actually produced by the recipient, normal mice were injected with 20 μg complexes formed at equivalence and they were bled 1, 2, 4, 8 and 24 hours later, respectively. These sera showed no detectable antibody. On the other hand, 66 units of free antibody, which were equivalent to 20 μg free BαA, showed a biological half life of about 2 days, when injected into normal mice. With these results

and the daily increasing response seen in Fig. 1, it was concluded that the antibody response seen with complexes was not passive but was the response actually and actively produced by the recipient.

In the next experiment, the relation between the ratio of Ag–Ab complexes and the primary antibody response was studied. Normal mice were injected with one of the following: (a) 10 μg BαA complexed with 6·6 units antibody (antigen excess), (b) 10 μg BαA complexed with 33 units antibody (equivalence), (c) 10 μg BαA complexed with 165 units antibody (antibody excess). As shown in Table 1, complexes at equivalence were most effective, then the antibody excess (one-fourth of the equivalent) and least the complexes formed in antigen excess (one-eighteenth of the equivalent). Therefore, in every subsequent experiment only complexes at equivalence were used.

TABLE 2

PRIMARY ANTIBODY RESPONSE BY Ag–Ab COMPLEXES FORMED *in vivo*

Normal mice injected with	Antibody titre	
	at 10th day	at 20th day
Anti-BαA antiserum then free BαA	37	300
Normal serum then free BαA	<5	<5

Eight to ten mice were used for the recipients of each group.

Antibody response as a function of variable doses of complexes was next studied. Normal mice were injected either with 0·02, 0·2, 2, 20 and 200 μg complexes or with the corresponding amounts of free BαA. The results, summarized in Fig. 2, show a clear linear relationship between the amount of complexes and the antibody response. Mice given 200 μg complexes were all found to be dead by the next day, probably with anaphylactic shock. All the mice given free BαA did not elicit any antibody response.

The complexes prepared *in vitro*, thus proved to be a greatly effective immunogen and this was also found with complexes formed *in vivo* in the next experiment.

Normal mice were injected either with 0.25 ml antiserum containing 66 units antibody or with 0.25 ml normal serum and 1 hour later they were challenged with 20 μg free BαA (Table 2). The mice receiving antibody then antigen showed a clear primary antibody response, though it was considerably less than that by complexes prepared *in vitro*. Therefore, it was proved that the preparation of complexes *in vitro* was directly related to their antigenic activity.

In the next set of experiments, how and why originally inactive BαA could become so strong an immunogen when combined with antibody were investigated.

PHAGOCYTOSIS AND DEGRADATION OF Ag–Ab COMPLEXES *in vivo* AND *in vitro*

Ag–Ab complexes are, in general, said to be phagocytosed more rapidly than free antigen (Weigle, 1961) and some phagocytes (macrophages and/or phagocytic reticular cells) are said to be very important for the antibody response (Fishman, 1961; Burnet, 1969).

Therefore, at first, whether this difference of antigen uptake by phagocytes was related to the difference of antibody response was tested. Normal mice were injected either with

20 μg ^{131}I-BαA or with 20 μg ^{131}I-complexes. They were bled 15, 30, 60 and 120 minutes later, respectively, and the radioactivity of each sample was measured to estimate the antigen eliminated from the blood circulation.

As is shown in Fig. 3, complexes were eliminated, and therefore phagocytosed, from the circulation more rapidly than free BαA with clear significance during earlier period. After

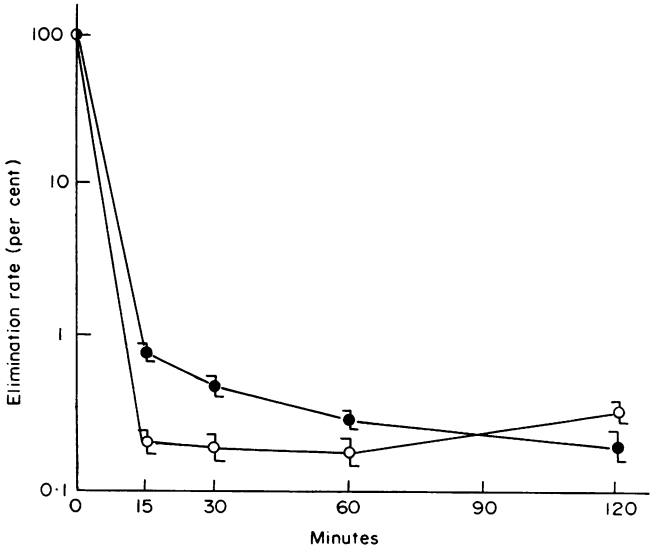

FIG. 3. Elimination curves of Ag–Ab complexes and free BαA from the circulation. Normal mice were injected i.v. with 20 μg ^{131}I-Ag–Ab complexes or 20 μg ^{131}I-free BαA. They were bled 15, 30, 60 and 120 minutes later, respectively and the radioactivity of each sample was measured. Open circles, complexes; solid circles, free BαA. Each symbol represents the mean value of six mice.

60 minutes, however, the elimination curve of complexes was gradually rising and at 120 minutes it was higher than that of free BαA, which was still disappearing from the circulation. These results suggested that complexes were not only phagocytosed but also degraded more rapidly than free BαA. This was confirmed in the next *in vitro* experiment, where the lymphoid cells, the actual participants for antibody response, were used.

Normal spleen and lymph node cell suspensions of 10^8 cells/ml were incubated *in vitro* either with 20 μg/ml ^{131}I-BαA or with 20 μg/ml ^{131}I-complexes. After 1 hour incubation at 37° they were washed five times with sufficient HBSS and finally resuspended in Eagle's MEM to give 10^8 cells/ml. A 1-ml portion of each suspension was used for measurement of radioactivity to estimate the amounts of antigen phagocytosed by the cells. The other portion of each was cultured *in vitro* for further 2 hours at 37°, then the supernatant of each cell culture was obtained by centrifugation at 1500 rev/min for 20 minutes.

After recentrifugation at 2500 rev/min for 30 minutes, a 1-ml portion of each supernatant was used for measurement of radioactivity to estimate the amounts of antigen degraded and excreted by the cells. The results are summarized in Table 3, in which it is clearly seen that complexes were not only phagocytosed but also degraded more rapidly than free BαA, which coincided well with the above *in vitro* observations. The ratio of phagocytosis of complexes versus free BαA was 2·6:1 and that of degradation was 3·2:1 in these experimental conditions. Thus, it was suggested that the recipient given free BαA would keep more antigen in its phagocytic cells than the recipient given complexes within very

TABLE 3

In vitro PHAGOCYTOSIS AND DEGRADATION OF Ag–Ab COMPLEXES OR FREE BαA BY NORMAL SPLEEN AND LYMPH NODE CELLS

Incubation of normal spleen and lymph node cells with*	Phagocytosis† (per cent)	Degradation of phagocytosed antigen‡ (per cent)
Ag–Ab complexes	5.13	20·0
Free BαA	1·96	6·25

* Normal spleen and lymph node cells (10^8 cells/ml) were incubated with 20 μg/ml ^{131}I-complexes or 20 μg/ml ^{131}I-BαA.

† After 1 hour incubation and thorough washing, the radioactivity of 1 ml of each 10^8 cells/ml was compared with that of original complexes or free BαA.

‡ Cells which had taken up complexes or free BαA (10^8 cells/ml) were cultured a further two hours and the radioactivity of 1 ml of each culture supernatant was compared with that of the corresponding cells before culture.

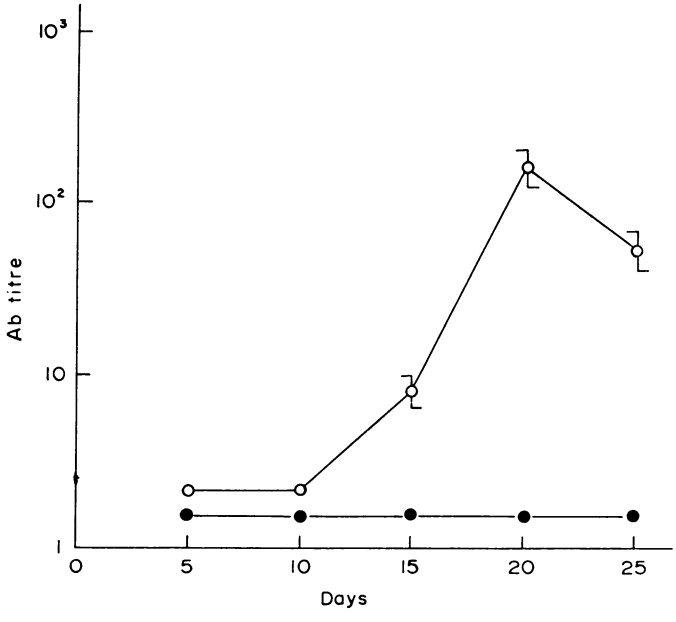

FIG. 4. Primary antibody response by non-irradiated mice inoculated with Ag–Ab complexes phagocytosed by normal spleen and lymph node cells. Normal spleen and lymph node cells were incubated *in vitro* with complexes or free BαA. After thorough washing, they were injected i.v. into normal non-irradiated recipients. Open circles, complexes phagocytosed by cells; solid circles, free BαA phagocytosed by cells. Each symbol represents the mean value of eight to ten mice.

early hours after administration and therefore the difference of immunogenicity between complexes and free BαA might not be due to the difference of the quantity of antigen phagocytosed by the cells.

PRIMARY ANTIBODY RESPONSE ELICITED BY THE Ag–Ab COMPLEXES PHAGOCYTOSED BY CELLS

The next question was whether these complexes phagocytosed by cells in normal spleen and lymph node cells were really effective for primary antibody response. To test this, 10^8/ml normal spleen and lymph node cells were incubated *in vitro* with 20 μg/ml BαA or 20 μg/ml complexes for 1 hour at 37°. After thorough washing, each of the two was resuspended in Eagle's MEM to give 2×10^7 cells/ml and 0·5 ml of each was injected into non-irradiated recipients. The animals were bled several times after treatment (Fig. 4). The cells which had phagocytosed complexes were indeed effective for primary antibody

TABLE 4

PRIMARY ANTIBODY RESPONSE BY IRRADIATED MICE INOCULATED WITH Ag–Ab COMPLEXES PHAGOCYTOSED BY CELLS PLUS NORMAL SPLEEN AND LYMPH NODE CELLS

Treatment of irradiated recipient (per mouse)	No. of cells	Antibody titre (at 20th day)
Ag–Ab complexes phagocytosed by cells	5×10^6	<5
Ag–Ab complexes phagocytosed by cells with normal spleen and lymph node cells	5×10^6 1×10^8	147
Free BαA phagocytosed by cells	5×10^6	<5
Free BαA phagocytosed by cells with normal spleen and lymph node cells	5×10^6 1×10^8	<5

Each titre represents the mean value of five to eight mice.

response. On the other hand, the cells which had phagocytosed free BαA were completely ineffective, though they had phagocytosed at least a third as much antigen as complexes (Table 3). These results provided further comfirmation for the above suggestion.

In a further experiment normal spleen and lymph node cells were treated as above and after washing they were resuspended in Eagle's MEM to give 10^7 cells/ml. 0·5 ml of each cell suspension was injected into 600 r X-irradiated recipients with or without 0·5 ml of 2×10^8/ml normal spleen and lymph node cells (Table 4). Only the recipients given cells fed complexes *plus* normal cells showed a primary antibody response, which suggested that the cells which had phagocytosed complexes themselves were not able to produce antibody.

MODE OF INTERACTION OF CELLS WITH PHAGOCYTOSED Ag–Ab COMPLEXES AND ANTIBODY-PRODUCING CELLS

To study this, normal spleen and lymph node cells (10^8 cells/ml) were incubated with 20 μg/ml complexes. After washing, they were resuspended in Eagle's MEM and 0·5 ml of 2×10^7 cells/ml were injected into normal non-irradiated recipients with antiserum containing 10 units antibody or with normal serum (Table 5). Suppression was clearly seen in the recipient given antibody and this suggested that the mode of the interaction between

the phagocytic cells and other lymphoid cells might be via immunogenic substances released from the former cells rather than via direct contact between them. Therefore, it might be possible to get such immunogenic substances *in vitro* on culturing cells which had taken up complexes for an appropriate period. This possibility was tested in the next experiment.

TABLE 5

ANTIBODY SUPPRESSION OF PRIMARY ANTIBODY RESPONSE BY NON-IRRADIATED MICE INOCULATED WITH Ag–Ab COMPLEXES PHAGOCYTOSED BY NORMAL SPLEEN AND LYMPH NODE CELLS

Treatment of non-irradiated recipient (per mouse)	No. of cells	Antibody titre (at 20th day)
Ag–Ab complexes phagocytosed by cells with antiserum containing 10 units antibody	1×10^7	<5
Ag–Ab complexes phagocytosed by cells with normal serum	1×10^7	590

Eight to ten mice were used for the recipient of each group.

PRIMARY ANTIBODY RESPONSE ELICITED BY THE CULTURE SUPERNATANT OF CELLS WHICH HAD PHAGOCYTOSED Ag–Ab COMPLEXES

Two bulk culture supernatants were collected from the culture fluids of normal spleen and lymph node cells phagocytosing either ^{131}I-BαA or ^{131}I-complexes. The details of treatments of the cells were the same as in the experiment of Table 3. After centrifugations twice, the two supernatants were ultrafiltered and dialysed against Eagle's

TABLE 6

PRIMARY ANTIBODY RESPONSE BY NORMAL MICE INJECTED WITH THE CULTURE SUPERNATANT FROM Ag–Ab COMPLEXES PHAGOCYTOSED BY NORMAL SPLEEN AND LYMPH NODE CELLS

Treatment of non-irradiated recipient (per mouse)	Antibody titre (at 20th day)
Supernatant from Ag–Ab complexes phagocytosed by cell cultures (200 ng)*	540
Supernatant from free BαA phagocytosed by cell cultures (200 ng)†	$<5^2$
Original Ag–Ab complexes (200 ng)	37
Original free BαA (200 ng)	<5

* The antigen dose in each supernatant was determined by comparing its radioactivity with that of original complexes or free BαA.

† The recipients of this supernatant were only three because of the shortage of this supernatant. In other groups, they were six to nine.

MEM overnight at 4°, then, by measuring radioactivity, adjusted with Eagle's MEM to give 400 ng/ml antigen, respectively. 0·5 ml of each supernatant was injected into normal non-irradiated recipients. Control mice were injected either with 0·5 ml of 400 ng/ml original complexes or with 0·5 ml of 400 ng/ml original free BαA. The results are summarized in Table 6. The supernatant of cells which had taken up complexes elicited a primary

antibody response but that of cells which had phagocytosed free BαA did not. Moreover, the response was more than ten times higher than that of the original complexes and this clearly excluded the possibility that the effectiveness of this supernatant might be due to residual complexes not removed by washing or which had merely attached to the cell membranes and had moved into the supernatant during the culture.

SECONDARY ANTIBODY RESPONSE ELICITED BY THE CULTURE SUPERNATANT OF Ag–Ab COMPLEXES PHAGOCYTOSED CELLS

In the next experiment the effectiveness of these supernatants for the secondary antibody response was studied. Primed spleen and lymph node cells of 10^8 cells/ml were incubated *in vitro* with one of the following: (a) 1 ng/ml supernatant of cells which had phagocytose compplexes, (b) 1 ng/ml supernatant of cells which had taken up free BαA, (c) 1 ng/ml original

TABLE 7

SECONDARY ANTIBODY RESPONSE OF PRIMED CELLS STIMULATED *in vitro* WITH THE CULTURE SUPERNATANT FROM Ag–Ab COMPLEXES PHAGOCYTOSED BY NORMAL SPLEEN AND LYMPH NODE CELLS

Incubation of primed cells with	Antibody titre (at 10th day)
Supernatant from Ab–Ab complexes phagocytosed by cell cultures	4070
Supernatant from free BαA phagocytosed by cell cultures	<5
Original Ag–Ab complexes	590
Original free BαA	1020
Nil	<5

Primed cells were obtained from the spleens and lymph nodes of the mice which had been immunized with BαA in Freund's incomplete adjuvant 2 weeks previously.

10^8 cells/ml were incubated with 1 ng/ml of each agent and 5×10^7 cells were transferred into 600 r X-irradiated recipients.

Each titre represents the mean value of six to nine mice.

complexes and (d) 1 ng/ml original free BαA. After 30 minutes incubation at 37°, they were washed five times with sufficient HBSS, then resuspended in Eagle's MEM to give 10^8 cells/ml. 0·5 ml of each suspension was injected into 600 r X-irradiated non-sensitized recipients, respectively. Control mice were injected with primed cells only. The results are summarized in Table 7. The substances in the supernatant of cells which had phagocytosed complexes were also effective for the secondary antibody response which was, in this case too, about 7 times higher than that given by the original complexes and four times that of free BαA. The supernatant of cells which had taken up free BαA failed to stimulate even primed cells and this seemed to indicate that the phagocytosed free BαA was degraded to the extent of losing almost all its immunogenicity. Considering that the substances in the supernatant of cells which had phagocytosed complexes were, on the contrary, strongly immunogenic and probably contained antigenic fragments, it would appear that the manner of degradation by phagocytes was different for complexes and free BαA.

COMPARISON OF Ag–Ab COMPLEXES PREPARED WITH HETEROLOGOUS RABBIT ANTIBODY WITH THOSE PREPARED WITH HOMOLOGOUS MOUSE ANTIBODY

The final experiment was carried out to test whether the complexes prepared with heterologous rabbit antibody were also immunogenic for primary antibody response.

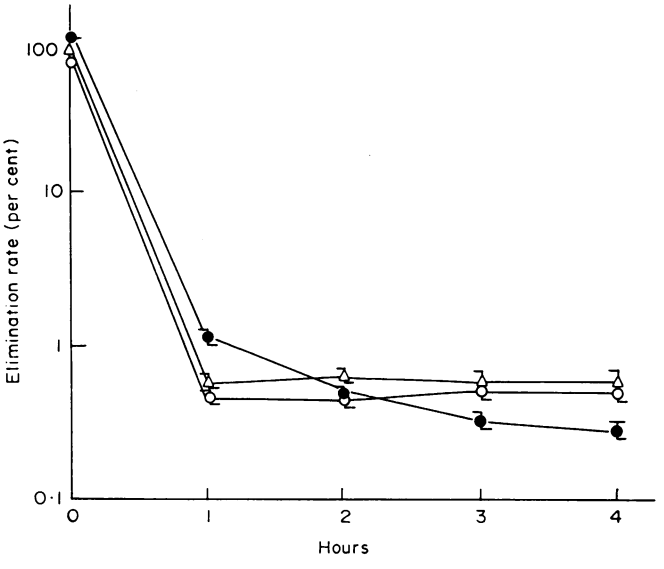

FIG. 5. Comparison of the elimination curves of Ag–Ab complexes prepared with homologous mouse or heterologous rabbit antibody from the circulation. Normal mice were injected i.v. with 20 μg ^{131}I-Ag–Ab complexes with rabbit antibody or with homologous mouse antibody or 20 μg ^{131}I-free BαA. They were bled 1, 2, 3 and 4 hours later, respectively, and the radioactivity of each sample was measured. Open circles, complexes with homologous antibody; open triangles, complexes with rabbit antibody; solid circles, free BαA. Each symbol represents the mean value of six mice.

TABLE 8

COMPARISON OF IMMUNOGENICITY BETWEEN Ag–Ab COMPLEXES PREPARED WITH HOMOLOGOUS ANTIBODY AND Ag–Ab COMPLEXES PREPARED WITH HETEROLOGOUS RABBIT ANTIBODY

Normal mice injected with	Antibody titre (at 20th day)
Ag–Ab complexes with homologous antibody (20 μg)	1110
Ag–Ab complexes with heterologous rabbit antibody (20 μg)	<5
Free BαA (20 μg)	<5

Normal mice were injected either with ^{131}I-complexes prepared with rabbit antibody or with ^{131}I-complexes prepared with homologous antibody or with ^{131}I-BαA. They were bled 1, 2, 3 and 4 hours later and the samples were used for the measurement of radioactivity, respectively (Fig. 5). Other normal mice were injected with 20 μg of each complexes to compare the antibody response (Table 8). As are shown in Fig. 5 and Table 8, the complexes with heterologous rabbit antibody did not elicit a primary antibody response but they were phagocytosed and degraded at the same rate as the complexes

with homologous antibody. These results suggested that the greater rate of degradation of complexes relative to free BαA was not itself important for immunogenicity. The source of antibody thus seemed to be important and heterologous, at least rabbit, antibody was not effective in the present experimental conditions.

DISCUSSION

In the present experiments it was clearly shown that originally inactive free BαA became strongly immunogenic when combined with homologous anti-BαA antibody and that phagocytes in normal spleen and lymph node cells played a very important role for this change of immunogenicity.

In basically similar experiments, though they studied the secondary response, Hamaoka *et al.* (1971) showed that the effective phagocytes are macrophages, that the antibody which was effective in increasing immunogenicity is probably not cytophilic and that complement is not important for this change of immunogenicity. Therefore, these issues will not be discussed here further.

Ag–Ab complexes are said to be phagocytosed more rapidly than free antigen (Weigle, 1961) and this was the case in the present experiments. This rapid phagocytosis itself, however, was not an important factor in increasing immunogenicity of complexes (Table 3, Fig. 4). Surprisingly, complexes were degraded also more rapidly than free BαA and this result strongly suggested that the effectiveness of complexes is not due to a quantitative difference of phagocytosed antigen but a qualitative difference of the degraded products. This was indeed the case as shown in Table 7, where the degraded products of complexes fed phagocytes proved to be an even stronger immunogen than the original complexes, not only for a primary antibody response but also for primed cells. These immunogenic products appeared to have antigenic fragments still capable of reacting with antibody (Table 5) and this was in contrast with the degraded products of fre BαA fed to phagocytes. The latter products seem to be degraded almost completely, probably to the level of amino acids as Ehrenreich and Cohn (1967, 1968) have reported, because they were ineffective not only for a primary response but even for primed cells (Table 6, Table 7).

Therefore, it seems logical to think that the virtue of antibody in Ag–Ab complexes will be expressed at the site of antigen degradation in phagocytes. It may not be, however, the mere protection of antigen against antigen degradation by covering antigen nor by hastening the degradation, because the complexes prepared with heterologous rabbit antibody, which was phagocytosed and degraded at roughly the same rate as the complexes with homologous antibody, was ineffective for the primary antibody response (Fig. 5, Table 8). Thus, it is highly likely that the manner of antigen degradation by phagocytes is different for the two complexes and this is probably true also for free BαA and complexes with homologous antibody (Fig. 4, Table 7).

Hamaoka *et al.* (1971) showed that the BαA-5*S* pepsin digested divalent antibody complexes were much less immunogenic than complete complexes and concluded that the Fc portion must play a part. Their result seems to be well explained by the above discussion that the manner of degradation of complexes would be different whether they were complete or they lacked the Fc portion.

Ag–Ab complexes prepared with rabbit antibody were also different in another respect. 50 μg complexes with homologous antibody caused lethal anaphylactic shock in all recipients

but even 150 μg complexes with rabbit antibody did not elicit any sign of hypersensitivity (unpublished data).

Though this may not be directly related to the antibody response, it strongly suggests that there is a qualitative difference between the two complexes and therefore it is perhaps not surprising that the complexes prepared with rabbit antibody were ineffective for the primary antibody response under the present experimental conditions.

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