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Antibody Formation by Transferred Peritoneal Cells and Spleen Cells of Mice

I. TRANSFER OF CELLS FROM IMMUNIZED NON-IRRADIATED DONORS TO SYNGENEIC RECIPIENTS WITH AND WITHOUT ANTIGEN

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Summary. Peritoneal cells and spleen cells from LAF_1 mice given three intraperitoneal immunizations of sheep red blood cells synthesized haemagglutinins after transfer to X-irradiated syngeneic recipients, either with or without a concomitant injection of antigen. Antibody formation by cells transferred with antigen resembled a secondary antibody response in intact animals. Haemagglutinins appeared rapidly and in high titre. Approximately 50 per cent of the antibodies were resistant to treatment with 2-mercaptoethanol.

Antibody formation by cells transferred without further exposure to antigen differed in several respects. Haemagglutinin titres were lower. Throughout the period of observation, only 6–25 per cent of the antibodies formed were mercaptoethanol-resistant. In recipients injected with spleen cells, antibodies appeared rapidly, suggesting that mature antibody-forming cells had been transferred. However, in recipients injected with peritoneal cells from the same donors, antibodies were detected only after a delay of several days, which suggested that mature antibody-forming cells had not been transferred. Haemagglutinins were synthesized equally well after transfer of all types of peritoneal cells or of a fraction consisting almost entirely of lymphoid cells. Recipients of peritoneal cells from donors which had been given either one intraperitoneal immunization or three intravenous immunizations had no or only low haemagglutinin titres, in contrast to recipients of cells from donors given three intraperitoneal immunizations.

These observations suggest that antibody formation in recipients of peritoneal cells only, i.e. without further stimulation by antigen, can be attributed to cells which are present in the peritoneal cavity of donors immunized repeatedly by the intraperitoneal route, but which are not part of the active antibody-synthesizing apparatus of the donors at the time of cell transfer. The development of these cells into antibody-forming cells can be detected only in an environment devoid of antigen and of mature antibody-forming cells. It is postulated that these cells are distinct from cells which respond on re-exposure to antigen.

INTRODUCTION

Immunologically functional cells can be isolated from their natural environment and can produce antibody after transfer to a suitable recipient. Any antibody detected in the serum of the recipient is then attributed to the transferred cells, provided that antibody

production by the recipient has been ruled out. The successful transfer of antibody formation depends on several factors, one of the most important being the type of cells employed. Lymphoid cells obtained from lymph nodes, spleen, thymus, circulating blood and the peritoneal cavity, have been reported to produce antibody after transfer to recipient animals. A detailed discussion of this subject can be found in the excellent review by Cochrane and Dixon (1962).

Two distinct approaches have been employed for the study of antibody formation by transferred cells. The one, used by the majority of investigators, is based on the ability of cells to give a primary or secondary response after transfer and antigenic stimulation in immunologically incompetent recipients. The other approach, used less widely, involves the transfer of cells either already engaged in antibody formation or able to develop into such cells without further contact with antigen.

The present study was undertaken to compare these two experimental approaches, with emphasis on antibody production by transferred peritoneal cells of immunized mice. Some of the data included here have been presented elsewhere (Kornfeld, 1967).

MATERIALS AND METHODS

Mice

Female LAF_1 mice (C57L female × A/He male) from our laboratory colony were used in all experiments. Mice used as recipients were between 12 and 16 weeks old, those used for immunization (cell donors) were generally 4–6 months of age.

X-Irradiation of recipient mice

A single total-body exposure of 600 r was delivered 24 hours before cell transfer. Irradiation factors were: 250 kVp, 15 mA, filters 0·5 mm Cu and 1 mm Al, HVL 1·49 mm Cu, TSD 40 in., dose rate 27·8 r/min. No deaths occurred as a result of this exposure.

Immunization of donor mice

Except when indicated otherwise, three intraperitoneal injections of 2×10^8 washed sheep red blood cells (SRBC) were given at weekly intervals. Haemagglutinin titres of donor mice were 2^9 or 2^{10}. After treatment with mercaptoethanol, titres were one $\log_2$ unit lower.

Cell harvest and transfer

At appropriate intervals after the final immunization, donor mice were killed by cervical dislocation. The free cells in the peritoneal cavity were washed out with Medium 199 containing 0·5 units heparin/ml. The cells were collected by centrifugation at 500 ***g*** for 15 minutes, washed twice and resuspended in Medium 199. Cells were counted in a haemacytometer. Viability was determined by trypan blue exclusion. Differential counts were made under phase contrast (Kornfeld and Greenman, 1966).

Donors of peritoneal cells also served as donors of spleen cells. Spleens were minced in Medium 199 containing 5 per cent newborn calf serum. The cells were passed through a nylon filter, centrifuged, washed twice and resuspended in Medium 199 plus serum. Viable cells were counted in a haemacytometer.

All cell transfers were made by the intraperitoneal route.* The exact number of viable

* Preliminary experiments had shown that the intraperitoneal and intravenous routes were equally effective.

cells transferred is given with the results. Every experiment was performed two or more times with highly reproducible results. Each of the curves shown in Figs. 1–4 represents the data from a single experiment, employing about five mice per group.

Haemagglutinin titrations

Serial bleedings of recipient mice were made from the retro-orbital plexus at intervals after cell transfer. Two-fold dilutions of individual sera in phosphate-buffered saline (pH 7·2) were made in disposable plastic trays. An equal volume (0·05 ml) of a 1 per cent suspension of SRBC was added to each cup. After gentle agitation, the trays remained undisturbed for 2 hours at room temperature. The titres recorded were the reciprocals of the highest serum dilutions, expressed as $\log_2$ units, showing characteristic agglutination patterns. The arithmetic mean of the $\log_2$ titres was calculated for each group. The standard errors of the means rarely exceeded 0·7 $\log_2$ units.

Treatment with 2-mercaptoethanol

Because larger quantities of serum were required, the blood from two or more recipient mice was pooled. Equal volumes of mouse serum and 0·2 M 2-mercaptoethanol (ME) in phosphate buffered saline (pH 7·2) were incubated in stoppered tubes for 1 hour at room temperature. Another aliquot of each serum was diluted in buffered saline. ME-treated and untreated sera were dialysed against large volumes of 0·02 M iodoacetamide overnight at +4° (Adler, 1965). Haemagglutination tests were performed immediately thereafter.

Antibody molecules resistant to treatment with ME were assumed to have a sedimentation constant of 7S. The difference between total and 7S antibody was considered to be the 19S fraction. It is recognized, however, that resistance to ME does not fully distinguish 7S from 19S antibodies, as some 7S haemagglutinins have been found to be sensitive to ME (Adler, 1965; Dietrich, 1966).

RESULTS

TRANSFER OF CELLS FROM IMMUNIZED DONORS WITHOUT INJECTION OF ANTIGEN

In order to determine the optimum time after immunization for cell transfer, groups of mice were killed 4, 7, 14, or 28 days after the third immunization with SRBC. Approximately $22–27 \times 10^6$ washed peritoneal cells were injected either into non-irradiated mice or into mice which had been exposed to 600 r 24 hours previously. No antigen was given to the recipients. It can be seen in Fig. 1 that serum antibody appeared earlier and attained initially higher levels in recipients of peritoneal cells collected 4 or 7 days after the third injection of sheep cells than in recipients of cells obtained 14 or 28 days after the last immunization. After the transfer of 4- or 7-day cells to irradiated mice, haemagglutinins were not detected for 3 days. Titres rose rapidly during the next few days and levelled off 2–3 weeks after cell transfer. After the transfer of 14- or 28-day cells, haemagglutinins were not detected for at least 7 days. Titres rose as rapidly as in recipients of 4- or 7-day cells and again levelled off. The mean haemagglutinin titres of all groups were at approximately the same level 6 weeks after cell transfer and remained essentially unchanged for 8 more months. By the end of 1 year, when the experiment was terminated, the titres had decreased only slightly (about one $\log_2$ unit).

In non-irradiated recipients of the same 4-, 7- and 14-day cell suspensions, haemagglutinin titres were lower during the first 4 weeks after cell transfer and the delay in the

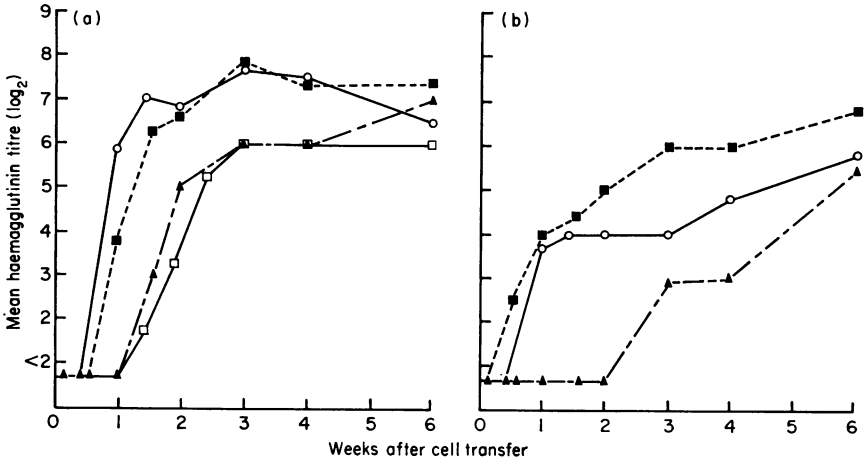

FIG. 1. Antibody formation by transferred peritoneal cells obtained at various times after immunization of the cell donors. (a) Irradiated recipients, (b) non-irradiated recipients.

Symbol	Time of transfer (days after third i.p immunization)	No. of cells transferred
○	4	27×10^6
■	7	26×10^6
▲	14	22×10^6
□	28	22×10^6

TABLE 1

HAEMAGGLUTININ TITRES OF IRRADIATED AND NON-IRRADIATED MICE AFTER TRANSFER OF PERITONEAL CELLS OBTAINED 14 DAYS AFTER THE THIRD IMMUNIZATION OF THE DONORS

No. of cells transferred	Day post transfer	Irradiated recipients		Non-irradiated recipients	
		No. of mice with detectable antibodies	Mean titre (log_2)	No. of mice with detectable antibodies	Mean titre (log_2)
115×10^6	3	0/5		0/3	
	7	0/5		0/3	
	10	4/5	3·1*	0/3	
	14	5/5	5·2	1/3	1·3*
	17	5/5	6·6	1/3	1·7*
	21	5/5	6·8	2/3	3·2*
	24	5/5	7·8	3/3	5·3
	28	5/5	7·8	3/3	5·7
23×10^6	3	0/3			
	7	0/3			
	10	1/3	1·7*		
	14	3/3	4·7		
	17	3/3	5·7		
	21	3/3	5·7		
	24	3/3	6·7		
	28	3/3	7·0		

* Sera negative in the lowest dilution tested (1:4) were assigned a log_2 titre of 0·5 for purposes of calculating the mean titre for the group.

appearance of antibody after transfer of 14-day cells was longer (2 weeks). However, mean haemagglutinin titres of the non-irradiated recipients were as high 6 weeks after cell transfer as those of irradiated recipients and also persisted essentially unchanged for 1 year.

The question arose whether the long delay in the appearance of antibodies after transfer of peritoneal cells obtained 14 or 28 days after immunization of the donors could be shortened by injecting larger numbers of cells. Peritoneal cells were collected from donor mice 14 days after the last immunization with SRBC and injected into irradiated and non-irradiated recipients at two dose levels, 115×10^6 and 23×10^6 viable cells per mouse. Haemagglutinins appeared at the same time in both groups of irradiated recipients. All

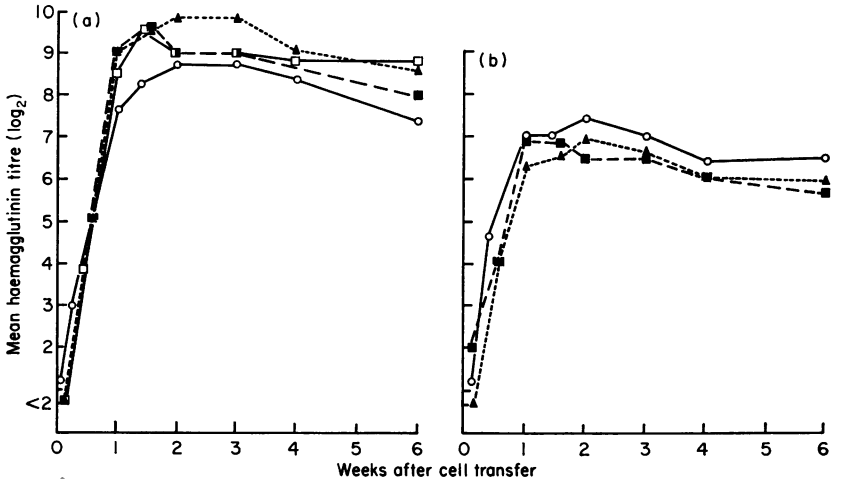

FIG. 2. Antibody formation by transferred spleen cells obtained at various times after immunization of the cell donor. (a) Irradiated recipients, (b) non-irradiated recipients.

Symbol	Time of transfer (days after third i.p. immunization)	No. of cells transferred
○	4	30×10^6
■	7	30×10^6
▲	14	20×10^6
□	28	22×10^6

irradiated mice had detectable antibodies 14 days after cell transfer. Haemagglutinins appeared more slowly and sporadically in non-irradiated recipients; all had demonstrable titres 24 days after cell transfer (Table 1). These results closely resembled those of an analogous experiment in which 22×10^6 peritoneal cells were transferred (Fig. 1). Thus, the delay in appearance of haemagglutinins after cell transfer was not shortened by increasing the cell dose five-fold. However, mean titres were slightly higher in those mice which had been given the larger quantity of cells.

For comparison, mice were injected with $20–30 \times 10^6$ washed spleen cells obtained from the same animals which had served as donors of peritoneal cells. No antigen was given to the recipients. As shown in Fig. 2, antibodies appeared at the same rates in

recipients of cells obtained 4, 7, 14 or 28 days after the last immunization. Haemagglutinins were always detected in 3 days, occasionally in 1 day. Peak titres were reached in 10–14 days. These kinetics were alike in irradiated and non-irradiated recipients. However, irradiated recipients attained higher titres than non-irradiated recipients of the same cell suspension.

Several additional variables and their effects on antibody formation by transferred peritoneal cells were studied. In all these experiments, a 4-day interval between the last immunization and the collection of peritoneal cells was employed. The results are summarized in Fig. 3. Higher haemagglutinin titres developed when a larger number of cells had been given. More antibody was produced by transferred peritoneal cells obtained from

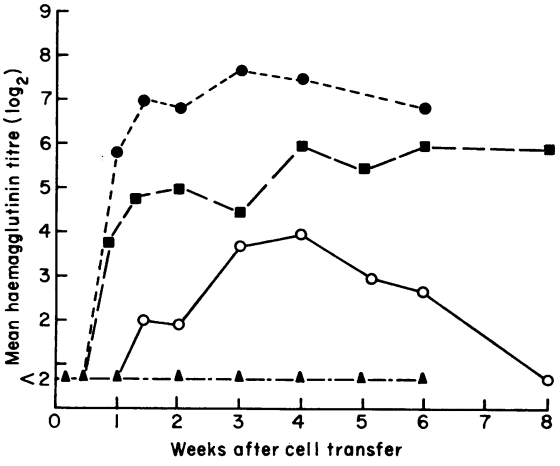

FIG. 3. Effect of route and number of immunizations of donor mice on amount of antibody formed by transferred peritoneal cells. All cell transfers 4 days after last immunization. Irradiated recipients.

Symbol	No. and route of immunizations	No. of cells transferred
●	3×SRBC i.p.	27×10^6
■	3×SRBC i.p.	12×10^6
▲	1×SRBC i.p.	$16{\cdot}6\times10^6$
○	3×SRBC i.v.	$22{\cdot}8\times10^6$

donor mice after three immunizations given intraperitoneally than intravenously. Haemagglutinins were found in recipients of peritoneal cells from mice given three intraperitoneal injections of SRBC, but were not detected in recipients of a similar number of peritoneal cells from donors immunized only once. This was true not only when donor cells were transferred 4 days after immunization, as shown in the figure, but also when peritoneal cells were transferred 7 or 14 days after a single immunization of the donors.

It was of interest to determine whether all the cell types found in the peritoneal cavity of mice were required for the formation of antibodies in the recipients. About 95 per cent of these cells are macrophages and small and medium lymphocytes (Kornfeld and Greenman, 1966). An aliquot of a suspension of peritoneal cells was incubated in plastic tissue

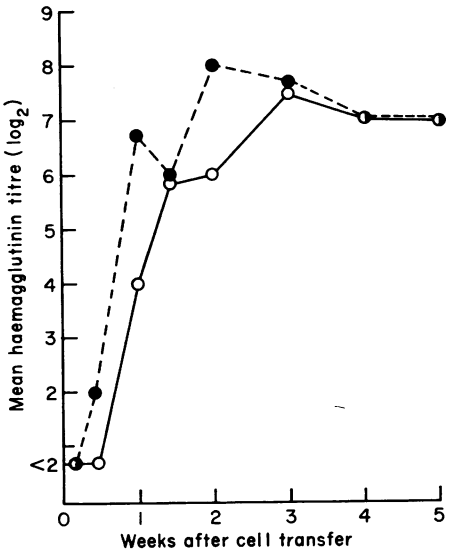

FIG. 4. Antibody formation by transferred peritoneal lymphocytes and mixed cell suspensions. ○, 3×10^6 macrophages + 18×10^6 lymphocytes; ●, 19×10^6 lymphocytes. Cell transfers 4 days after third i.p. immunization. Irradiated recipients.

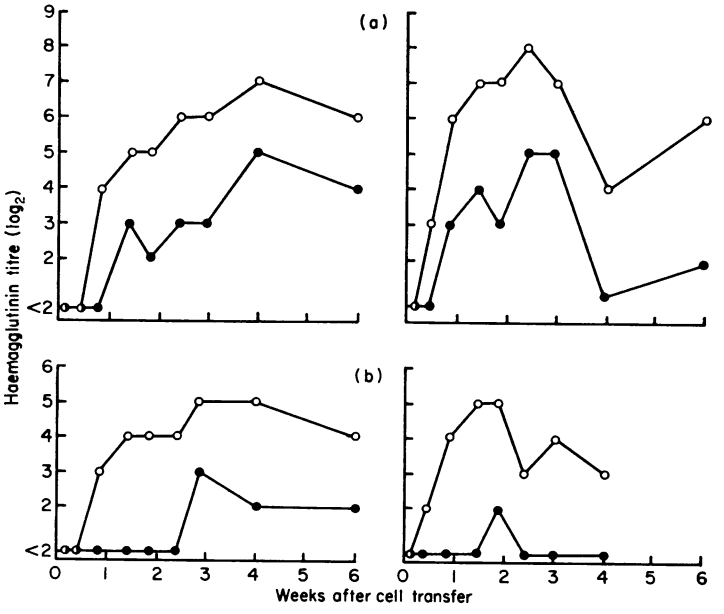

FIG. 5. Total and mercaptoethanol-resistant antibody formed by transferred peritoneal cells and spleen cells. Left-hand graphs: peritoneal cells, 22×10^6 cells transferred 4 days after third i.p. immunization. Right-hand graphs: spleen cells, 15×10^6 cells transferred 4 days after third i.p. immunization. (a) Irradiated recipients, (b) non-irradiated recipients. ○, Total haemagglutinins; ●, mercaptoethanol-resistant haemagglutinins.

culture flasks for 2 hours at 37°. The macrophages adhered to the bottom of the vessel and the supernatant consisted almost entirely of lymphocytes (99+ per cent). Haemagglutinin titres of mice given either this lymphocyte fraction or the original cell suspension are shown in Fig. 4. Antibody titres were at least as high in recipients of the lymphocyte fraction as in those receiving the whole cell suspension. Haemagglutinins remained undiminished for the duration of the experiment (6 months).

Washed peritoneal cells from immunized donors were exposed to 1000 r *in vitro* immediately before injection into irradiated and non-irradiated recipients. Haemagglutinin

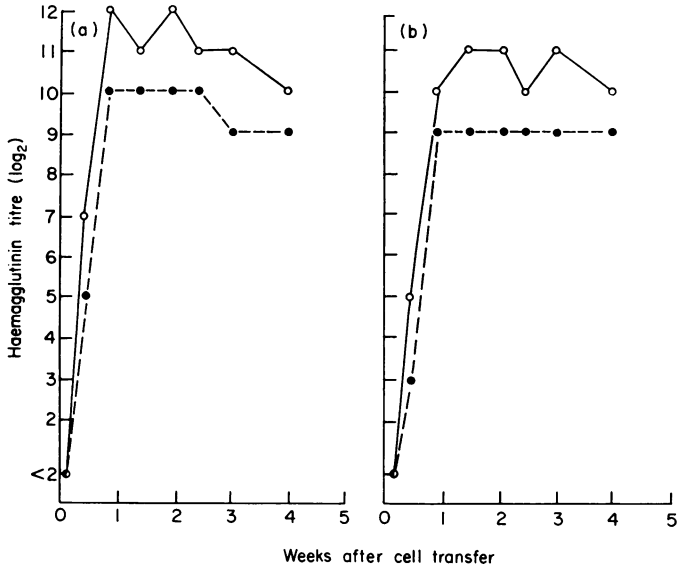

FIG. 6. Total and mercaptoethanol-resistant antibody formed in irradiated recipients by transferred peritoneal cells and spleen cells restimulated with antigen. (a) Peritoneal cells, 26×10^6 cells transferred 4 days after third i.p. immunization; (b) spleen cells, 17×10^6 cells transferred 4 days after third i.p. immunization. ○, Total haemagglutinins; ●, mercaptoethanol-resistant haemagglutinins.

titres of mice which had received about 22×10^6 irradiated cells never exceeded 2^3. In contrast, after transfer of a comparable number of unirradiated cells, haemagglutinin titres were 2^6–2^7 in irradiated recipients, 2^5–2^6 in non-irradiated recipients.

In order to determine the species of antibody produced by transferred peritoneal cells and spleen cells, additional experiments like those presented in Figs. 1 and 2, employing only the 4-day interval, were performed. Two mice from each group were killed at each bleeding. An aliquot of each serum pool was treated with ME. Haemagglutinin titres of ME-treated and untreated sera are shown in Fig. 5. In irradiated recipients, titres of ME-resistant (7S) haemagglutinins were 2–4 log_2 units lower than total antibody titres. In non-irradiated recipients, no or only very low levels of 7S antibodies could be detected.

TRANSFER OF CELLS FROM IMMUNIZED DONORS FOLLOWED BY INJECTION OF ANTIGEN

Experiments were carried out essentially like those described in the preceding section. Peritoneal cells and spleen cells, obtained from the same donors 4 days after the third immunization, were transferred to irradiated recipients. However, immediately following

injection of the donor cells, the recipients were also given 2×10^8 SRBC intraperitoneally. At appropriate intervals, two mice from each group were killed and their pooled sera were tested before and after treatment with ME. The results are shown in Fig. 6. Agglutinins in high titre were produced without delay in both groups of recipients. Titres of 7S haemagglutinins were 1–2 $\log_2$ units lower than total antibody titres. A similar experiment, employing peritoneal cells and spleen cells obtained 28 days after immunization of the donors, gave results essentially like those shown in Fig. 6.

Peritoneal cells collected 7 days after a single immunization were transferred to irradiated recipients injected also with SRBC. Again, haemagglutinins appeared rapidly

TABLE 2

TOTAL AND ME-RESISTANT HAEMAGGLUTININS OF IRRADIATED MICE AFTER INJECTION OF SRBC AND TRANSFER OF PERITONEAL CELLS FROM DONORS GIVEN ONLY A SINGLE IMMUNIZATION

No. of cells transferred	Day post transfer	Titre ($\log_2$)	
		Total	ME-resistant
22×10^6	3	4	<1
	6	8	7
	10	8	8
	14	8	7
	21	9	7

TABLE 3

TOTAL AND ME-RESISTANT HAEMAGGLUTININS OF IRRADIATED MICE AFTER INJECTION OF SRBC AND TRANSFER OF PERITONEAL CELLS FROM NON-IMMUNIZED DONORS

No. of cells transferred	Day post transfer	Titre ($\log_2$)	
		Total	ME-resistant
17×10^6	3	<2	
	6	<2	
	10	5	<1
	14	5	<1
	21	7	5

(Table 2), but titres were lower than in recipients of a comparable number of peritoneal cells from donors given three immunizations. Haemagglutinins detected 3 days after transfer were sensitive to ME, thereafter titres of ME-treated sera were about one $\log_2$ unit lower than those of untreated sera.

In order to differentiate a secondary response by transferred cells exposed to antigen in the recipients (Fig. 6, Table 2) from a primary response, SRBC and peritoneal cells from non-immunized donors were injected into mice irradiated (600 r) 24 hours earlier. Haemagglutinins (19S) were first detected 10 days after cell transfer, the earliest 7S haemagglutinins were found 21 days after transfer (Table 3).

It is now well established that animals immunized 1 day after exposure to X-rays make little or no antibody for several days or weeks (Taliaferro, Taliaferro and Jaroslow, 1964).

Nevertheless, it was necessary to ascertain for how long the recipients' own cells could not respond to the antigen given at the time of cell transfer. Mice were exposed to 600 r and injected intraperitoneally with 2×10^8 SRBC 24 hours later. No donor cells were given. Haemagglutinins, which were ME-sensitive, were first detected 28 days after immunization. Sera obtained one or more weeks after the onset of antibody production contained both 19S and 7S haemagglutinins (Table 4).

TABLE 4

TOTAL AND ME-RESISTANT HAEMAGGLUTININS OF MICE IMMUNIZED WITH SRBC 24 HOURS AFTER EXPOSURE TO 600 r

Day post immunization	Titre ($\log_2$)	
	Total	ME-resistant
14	<2	
21	<2	
24	<2	
28	4	<1
32	5	<1
35	6	3
45	6	3
56	5	2

DISCUSSION

These studies confirm and extend the findings of other investigators (Cochrane and Dixon, 1962) by showing that peritoneal cells or spleen cells from immunized mice produced antibodies after transfer to syngeneic X-irradiated recipients injected simultaneously with antigen. In these recipients, the characteristics of haemagglutinin formation closely resembled those of a secondary response to SRBC in intact non-irradiated mice. Our experiments also demonstrated that peritoneal cells or spleen cells from immunized donors were able to produce antibodies in syngeneic recipients not injected with antigen. However, some of the characteristics of haemagglutinin formation by cells transferred without antigen were distinct from those of a secondary and also from a primary response in intact animals. The haemagglutinins produced by cells transferred to irradiated recipients with and without a concomitant injection of SRBC differed in quantity, quality, and, in the case of peritoneal cells, also in the time of their appearance.

Antibody titres were higher in irradiated mice given peritoneal cells or spleen cells from immunized donors plus antigen (Fig. 6) than in mice given cells only (Fig. 5a). This was even more striking after the transfer of peritoneal cells from donors immunized only once (Table 2, Fig. 3). It is likely that in antigen-injected recipients, immunologically committed donor cells differentiated and/or proliferated rapidly into antibody-forming cells. Consequently, if more cells synthesized antibodies, one would expect titres to have been higher. This interpretation is in accord with the findings of Perkins, Robinson and Makinodan (1961), who showed that the amount of antibody produced was a linear function of the number of cells engaged in antibody formation.

The relative amounts of 19S and 7S antibody formed also differed in irradiated mice injected with cells from immunized donors plus antigen from mice injected with the same suspensions of donor cells only. In recipients injected with antigen, 25–50 per cent of the

haemagglutinins were resistant to treatment with ME (Fig. 6). Similar amounts of ME-resistant (7S) antibody were produced by our hyperimmunized donor animals. However, in mice injected with donor cells but not with antigen, only 6–25 per cent of the antibody formed was 7S (Fig. 5a). This fraction was smaller than that formed by the same cell suspensions after transfer with antigen, and also smaller than that formed by the hyperimmunized donors of these cells. Small amounts of 7S antibody are characteristic of an early primary response, but in the course of a primary response in intact animals, the fraction of 7S antibody increases after a few days (Uhr and Finkelstein, 1967). This increase, however, did not occur in recipients of transferred cells, since the low percentage of 7S haemagglutinins remained essentially unchanged over a period of several weeks. Blinkoff (1966) recently reported that mouse spleen cells obtained 2 or 6 days after a primary intravenous injection of flagellin produced only ME-sensitive (19S) agglutinins after transfer to recipients without antigen injection. These antibodies were not followed during the 1st week by 7S agglutinins, in contrast to the sequence observed in intact immunized mice.

It has been shown that most antibody-forming cells synthesize either 19S or 7S antibodies and that only an occasional cell produces both (Nossal, Szenberg, Ada and Austin, 1964). From this one may deduce that the ratio of 19S to 7S antibody in the serum depends on the ratio of cells which synthesize the two species of antibody. Thus, our observations may be interpreted to indicate that the cells which synthesize 19S and 7S antibodies exist in similar proportions in immunized donors and in recipients of their peritoneal cells or spleen cells plus antigen. However, this proportion is different in recipients of peritoneal cells or spleen cells only.

It is of particular interest to examine the time of appearance of antibodies after transfer of cells to irradiated recipients which had or had not been given SRBC. In antigen-injected recipients, haemagglutinins were detected 3 days after the transfer of either peritoneal cells or spleen cells (Fig. 6). In recipients not given antigen, haemagglutinins were also detected 3 days after the transfer of spleen cells (Fig. 2), but after the transfer of peritoneal cells, the appearance of haemagglutinins was delayed for 6–14 days, depending on the time interval between the last immunization of the donors and collection of their cells (Fig. 1). The latter results confirm those of Weiler (1964) who transferred peritoneal cells from mice immunized with bacteriophage and observed delayed antibody synthesis in the recipients.

It is highly probable that part of the antibody detected in antigen-injected recipients shortly after cell transfer was synthesized by donor cells which had matured rapidly into antibody-forming cells as a result of re-exposure to antigen in the recipients. It is also possible that part of the early antibody was produced by antibody-forming cells which had matured in the donors and continued to function in the recipients. Whether or not such cells did participate in the synthesis of haemagglutinins may be deduced from the experimental results obtained in recipients not given antigen, where a secondary response to SRBC presumably did not take place. Thus, the rapid appearance of haemagglutinins after transfer of spleen cells only strongly suggests that functional antibody-forming cells had been transferred. On the other hand, the delay in appearance of haemagglutinins after transfer of peritoneal cells only suggests that very few, if any, cells actively producing antibodies had been transferred.

Some experimental evidence (Kornfeld, unpublished results) may be cited in support of our speculations that cells actively forming antibodies were absent from the peritoneal

cavity of immunized donor mice. Pyroninophilic cells were not observed in suspensions of peritoneal cells obtained from LAF_1 mice at various times after intraperitoneal immunization with SRBC and all attempts to detect plaque-forming cells by the method of Jerne and Nordin (1963) were unsuccessful.

A puzzling observation was the 6- to 7-day delay in appearance of antibodies in irradiated recipients of peritoneal cells obtained 4 or 7 days after the last immunization of the donors and the 10- to 14-day delay when the donor cells had been obtained 14 or 28 days after immunization. If the length of the delay simply represented the time necessary for the synthesis of detectable amounts of antibody by varying numbers of transferred antibody-producing cells, the delay could have been shortened by injecting more donor cells. However, a five-fold increase in the number of transferred peritoneal cells obtained 14 days after the last immunization of the donors did not reduce the time of appearance of antibodies in the recipients (Table 1). Thus, it is likely that a definite time period was required for the differentiation of certain donor cells into antibody-forming cells. We postulate, therefore, that immunologically determined cells were transferred which differentiated and/or proliferated in the recipients into antibody-forming cells without requiring contact with antigen.

If cells capable of forming antibodies were not present in the suspensions of peritoneal cells from immunized donors but developed only after transfer to recipients without further contact with antigen, the question arises concerning the nature of the cells which developed into antibody-forming cells. The successful transfer of antibody formation with a peritoneal cell suspension consisting almost entirely of cells classified morphologically as lymphocytes (Fig. 4) strongly suggests that the immunological activity resided in cells belonging to the lymphoid series. Weiler and Weiler (1965) also showed that immunological activity was associated with the lymphocyte fraction of the peritoneal cell population.

Another question is whether the number of cells in the peritoneal cavity capable of developing into antibody-forming cells in recipients not given antigen was influenced by the immunization schedule. We found that the route and number of immunizations of the donor mice did markedly affect the ability of their peritoneal cells to synthesize antibody after transfer. Intraperitoneal immunization with SRBC was superior to intravenous immunization, three intraperitoneal injections were far superior to one (Fig. 3). These observations should be contrasted with the findings of Makinodan and Gengozian (1958) who reported that the route of injection of SRBC had only relatively small effects on the amount of serum antibody produced in intact mice. In our experiments also, serum haemagglutinin titres were the same whether donor mice had been immunized intraperitoneally or intravenously. Furthermore, serum titres of donor mice given three intraperitoneal immunizations were only 1–2 $\log_2$ units higher than those of donors immunized but once.

An interesting observation was that haemagglutinin titres of recipients not given antigen were essentially undiminished 1 year after the transfer of peritoneal cells from immunized donors. This raised the question of whether the original antibody-forming cells which had developed in the recipients were still functioning or whether they had been replaced by younger cells. In case of the latter alternative, did the new antibody-forming cells arise from transferred donor cells or from their progeny? These questions cannot be answered from the data available at this time.

When immunologically active cells were transferred without further exposure to antigen, it was possible to compare antibody formation by these cells in irradiated and non-irradi-

ated recipients. Haemagglutinin titres were almost always higher in irradiated mice (Figs. 1, 2 and 5, and Table 1). This observation, also made by other investigators, has been explained in terms of a greater proliferation stimulus in irradiated recipients or on the basis of the availability of more space for proliferating lymphoid cells in the depleted organs of irradiated animals (Cochrane and Dixon, 1962). Furthermore, the delay in the appearance of haemagglutinins after the transfer of peritoneal cells obtained 14 days after immunization of the donors was not as long in irradiated as in non-irradiated recipients (Fig. 1 and Table 1). This may also have been due to a greater proliferation stimulus or more available space for the proliferating cells. However, it should be pointed out that the length of the delay could not be shortened in either group of recipients by injecting larger numbers of donor cells. Thus, it would seem that the characteristics of antibody formation by cells transferred without antigen did not differ, except quantitatively, in irradiated and non-irradiated recipients.

When discussing antibody formation by cells transferred to recipients without exposure to antigen, it is necessary to rule out the possibility that antigen carried along with donor cells initiated antibody production by cells of the recipients. If this had been the case, only non-irradiated recipients could have responded during the first 3 weeks after cell transfer. Furthermore, antibody titres should have been comparable in recipients of a peritoneal cell suspension irradiated *in vitro* and of a suspension of fully viable cells, as the amount of antigen associated with each would have been equal. Since our results did not fulfill these conditions, it was concluded that any antigen which may have been associated with the donor cells was insufficient to initiate a response by the cells of the recipients, and that all antibody detected in the recipients was produced by the transferred donor cells. Harris, Harris and Farber (1954) and Weiler (1964) drew the same conclusions from their more extensive control experiments.

Collectively, several observations made in the present study—the low percentage of 7S antibody formed, the delay in the appearance of antibodies, and the importance of repeated intraperitoneal immunization of the cell donors—strongly suggest that antibody formation by peritoneal cells transferred to suitable recipients without antigen differs from antibody formation by cells transferred with antigen and from antibody formation in intact immunized animals. It is our belief that the former can be attributed to the immunological activity of certain cells which are present in the peritoneal cavity of donors immunized by the intraperitoneal route, but which are not active participants in the antibody-forming apparatus of the donor mice at the time of cell transfer. The ability of these cells to develop into antibody-forming cells can be detected only in an environment devoid of antigen and of mature antibody-forming cells. Weiler (1964) referred to such cells as 'determined'. Makinodan and Albright (1967) suggested that 'determined' cells are a third type of progenitor cell, distinct from those involved in the primary and secondary responses to antigen. Data will be presented in a subsequent communication indicating that 'determined' cells and cells active in a secondary response to antigen differ in sensitivity to X-irradiation.

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REFERENCES

ADLER, F. L. (1965). 'Studies on mouse antibodies. I. The response to sheep red cells.' *J. Immunol.*, **95**, 26.

BLINKOFF, R. C. (1966). 'γM and γG antibodies in mice: Dissociation of the normal immunoglobulin sequence.' *J. Immunol.*, **97**, 736.

COCHRANE, C. G. and DIXON, F. J. (1962). 'Antibody production by transferred cells.' *Advanc. Immunol.*, **2**, 205.

DIETRICH, F. M. (1966). 'The immune response to heterologous red cells in mice.' *Immunology*, **10**, 365.

HARRIS, S., HARRIS, T. N. and FARBER, M. B. (1954). 'Studies on the transfer of lymph node cells. I. Appearance of antibody in recipients of cells from donor rabbits injected with antigen.' *J. Immunol.*, **72**, 148.

JERNE, N. K. and NORDIN, A. A. (1963). 'Plaque formation in agar by single antibody-producing cells.' *Science*, **140**, 405.

KORNFELD, L. (1967). 'Transfer of antibody production by peritoneal cells from irradiated and non-irradiated immunized mice.' (Abstract). *Radiat. Res.*, **31**, 568.

KORNFELD, L. and GREENMAN, V. (1966). 'Effects of total-body X-irradiation on peritoneal cells of mice.' *Radiat. Res.*, **29**, 433.

MAKINODAN, T. and ALBRIGHT, J. F. (1967). 'Proliferative and differentiative manifestations of cellular immune potential.' *Progr. Allergy*, **10**, 1.

MAKINODAN, T. and GENGOZIAN, N. (1958). 'Primary antibody response to a distantly related heterologous antigen during maximum depression period after varying doses of X radiation.' *J. Immunol.*, **81**, 150.

NOSSAL, G. J. V., SZENBERG, A., ADA, G. L. and AUSTIN, C. M. (1964). 'Single cell studies on 19S antibody production.' *J. exp. Med.*, **119**, 485.

PERKINS, E. H., ROBINSON, M. A. and MAKINODAN, T. (1961). 'Agglutinin response, a function of cell number.' *J. Immunol.*, **86**, 533.

TALIAFERRO, W. H., TALIAFERRO, L. G. and JAROSLOW, B. N. (1964). *Radiation and Immune Mechanisms.* Academic Press, New York.

UHR, J. W. and FINKELSTEIN, M. S. (1967). 'The kinetics of antibody formation.' *Progr. Allergy*, **10**, 37.

WEILER, E. (1964). 'Delayed antibody synthesis in mice after transfer of immune peritoneal fluid cells.' *Immunology*, **7**, 197.

WEILER, I. J. and WEILER, E. (1965). 'Association of immunologic determination with the lymphocyte fraction of the peritoneal fluid cell population.' *J. Immunol.*, **95**, 288.