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Studies on the Immune Reconstitution of Sublethally Irradiated Mice by Peritoneal Macrophages

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Summary. Studies were performed on the reconstitution of the immune response to *Shigella paradysenteriae* and sheep erythrocytes in sublethally irradiated mice by the injection of normal peritoneal macrophages with and without pre-incubation with antigen. The response to *Shigella* was found to be less radiosensitive than the anti-SRBC response. Sublethally irradiated mice (550 r) injected with peritoneal macrophages from normal donors pre-incubated with *Shigella* antigen or injected simultaneously with macrophages and *Shigella* evinced a strong anti-*Shigella* response. No such reconstitution of the immune response against SRBC was observed, even when lower irradiation doses and phagocytosis-enhancing incubation media were used. Possible reasons for the different response to *Shigella* and SRBC are discussed.

INTRODUCTION

In recent years much information has accrued suggesting that macrophages process antigen for the immune response (Fishman, 1961; Fishman and Adler, 1963; Askonas and Rhodes, 1965; Adler, Fishman and Dray, 1966; Gottlieb, Glisen and Doty, 1967). After initial contact with antigen, the macrophage intermediary is believed to transfer an informational signal to the immune competent cell, which then proceeds to produce specific antibody. The nature of this informational signal is still an open question. It remains to be determined whether the macrophage processes the antigen and presents it to the lymphocyte in an immunogenic form, possibly of an RNA–antigen complex (Askonas and Rhodes, 1965; Gottlieb *et al.*, 1967) or whether it can also transfer a template for the synthesis of immunoglobulin with specific reactivity (Adler *et al.*, 1966).

Experiments were performed in this laboratory, designed to test whether the processing of antigen by macrophages is an essential step in the induction of antibody formation (Gallily and Feldman, 1966, 1967; Feldman and Gallily, 1968). These have shown that sublethally irradiated mice were immunologically reconstituted to evince an anti-*Shigella* response if they were provided with unirradiated peritoneal macrophages preincubated *in vitro* with alcohol-killed *Shigella paradysenteriae* (Gallily and Feldman, 1966, 1967; Feldman and Gallily, 1968). Lethally irradiated mice treated in the same manner were unable to evince the anti-*Shigella* response. However, if they were reconstituted with normal lymphocytes as well as macrophages, an immune response ensued (Feldman and Gallily, 1968). From these and other experiments, it was concluded that the macrophage is a necessary intermediary in the immune response to *Shigella*, and that damage to this cell is a major contributor to immune suppression of the sublethally irradiated mouse. We

proposed to test whether the capacity of macrophages from normal donors to confer immune reactivity on X-irradiated animals is a general phenomenon and to further investigate the nature of the information transferred by the macrophage. It was our intention to develop this system using a second antigen (sheep erythrocytes) and a haemolytic plaque assay (Jerne, Nordin and Henry, 1963) for cells producing 19S and 7S antibody (Dresser and Wortis, 1965; Sterzl and Riha, 1965), as well as antibody carrying specific allotype markers (Weiler, Melletz and Breuninger-Peck, 1965). The present report deals with the comparison between the *Shigella* and the erythrocyte systems.

MATERIALS AND METHODS

Animals

$(BALB/c \times DBA_2)F_1$ male mice (CD_2), 12 weeks old, were used as both donors and recipients in all experiments.

Antigens

Sheep erythrocytes (SRBC) were maintained in Alsever's solution (Kabat and Mayer, 1961) at 4°. Before use the SRBC were washed three times in saline (0·9 per cent NaCl). Isotonic suspensions of haemolysed SRBC ('ghosts') were produced by mixing 1 part packed SRBC with 4 parts distilled water; after shaking well, the haemolysate was allowed to stand at room temperature for 10 minutes and then reconstituted to physiological tonicity with 5 parts twice-concentrated Hanks's balanced salt solution. One-half a millilitre of the suspension of 'ghosts' contained the equivalent of 10^9 intact SRBC.

Whole, alcohol-killed *Shigella paradysenteriae*, prepared according to the method of Harris, Harris and Farber (1954), were maintained at 4° in a standard 10 per cent stock solution.

X-irradiation

Mice in leucite containers were irradiated with a General Electric Maximar III X-ray machine (20 kV, 15 mA), at a target distance of 50 cm (dose rate 65 r/min) with 0·5 mm Cu and 1 mm Al filters. The dose of total-body irradiation is indicated for each experiment described in 'Results'.

Macrophages

Macrophages were obtained as described by Gallily and Feldman (1967). Four days after a single i.p. injection of 3 ml thioglycolate medium (Difco), the peritoneal cavities of donor mice were washed with 8–10 ml phosphate buffered saline containing 100 units penicillin, 50 μg streptomycin, and 5 U.S.P. units heparin/ml. As described (Gallily and Feldman, 1967), the cells were counted, washed once and resuspended in Hanks's solution.

In vitro *incubation conditions*

The following incubation mixtures were prepared in 250-ml Erlenmeyer flasks: (1) 150×10^6 macrophages in 20 ml of 0·005 per cent *Shigella* suspension in Hanks's solution; (2) 150×10^6 macrophages plus 10^{10} SRBC, or 5 ml 'ghosts', in a final volume of 20 ml with Hanks's solution; (3) 150×10^6 macrophages plus 10^{10} SRBC, or 5 ml 'ghosts', in a final volume of 20 ml with normal serum or antibody, in Hanks's; and (4) 150×10^6 macrophages plus 5 ml 'ghosts' in 20 ml final incubation mixture of 0·005 per cent

Shigella suspension in Hanks's. All incubations were performed for 1 hour at 37° on a shaking tray. During this incubation period, a constant pH was maintained at 7·3. After incubation, the macrophage suspensions were washed three times with a minimum of 120 volumes cold Hanks's solution to remove free antigen and serum.

Immunization

The final washed pellet of macrophages was resuspended in Hanks's solution at a concentration of 30×10^6 cells/ml and injected intraperitoneally, in 0·5-ml aliquots, to sublethally irradiated recipient mice 48 hours after irradiation. In experiments in which the macrophages were not preincubated, they were made up to the required cell dosage of 15×10^6 cells/0·5 ml of the appropriate dilution of antigen in Hanks's solution, plus antisera if required. This mixture was then injected i.p. into recipient mice irradiated 48 hours previously. Individual normal or pre-irradiated control mice were immunized intraperitoneally with either 10^9 SRBC, 0·5 ml 'ghosts' or 0·1 ml of a 0·1 per cent suspension of *Shigella* in saline.

Sera used for incubation with macrophages

The following sera were maintained at −20° until use: CD_2 male normal serum; C57BL male normal serum; CD_2 male anti-sheep RBC serum obtained 5 days after a single i.p. injection of 10^9 SRBC ($1/\log_2$ agglutinin titre = 10). This serum is denoted as 5-day antiserum; CD_2 male anti-SRBC serum obtained 11 days after a single i.p. immunization with 10^9 SRBC ($1/\log_2$ agglutinin titre = 16). This serum is denoted as 11-day antiserum. Dilutions of sera and antisera used are indicated for each experiment.

Serology

On days 6 and 8 after immunization with antigen, or after inoculation with macrophages, mice were bled from either the retro-orbital sinus or a tail vein. Titrations of sera for anti-*Shigella* agglutinins were performed as reported by Gallily and Feldman (1967). For the detection of anti-SRBC agglutinins, 0·1 ml of a 1 per cent suspension of three times washed SRBC were added to serial two-fold dilutions of 0·1 ml serum in saline, shaken well, incubated at 37° for 1 hour, and then left at room temperature overnight. Titres were read by sedimentation pattern and shaking of the tubes.

Cytology

Smears of cell suspensions before and after incubation were stained with Lepehne stain and Giemsa. In experiments in which RBC 'ghosts' were used, parallel incubation mixtures with whole SRBC were set up so that phagocytosis would be clearly visible. The percentage of macrophages in active phagocytosis was determined for all experiments.

RESULTS

Preliminary tests revealed that the C57BL strain of mice, used by Gallily and Feldman (1967), was unsuitable for experiments with SRBC owing to its high level of natural anti-SRBC haemagglutinins. The CD_2 strain of mice was, therefore, chosen. As with the C57BL mice, a maximum yield of macrophages in the peritoneal exudate of CD_2 mice was obtained 4 days after an intraperitoneal injection of 3 ml thioglycolate medium.

An initial series of experiments was designed to determine whether peritoneal macrophages from normal mice, preincubated with SRBC, can restore the immune response of sublethally irradiated mice to this antigen. Recipient mice were irradiated with a dose of 550 r. Forty-eight hours later, macrophages from normal donor mice were incubated *in vitro* with whole SRBC. After the incubation, the macrophage-RBC mixture was gently centrifuged once. The SRBC were selectively lysed by dispersing the pellet in a small

TABLE 1

RESPONSE OF SUBLETHALLY IRRADIATED (550 r) CD_2 MICE TO SRBC FOLLOWING INOCULATION WITH PRE-INCUBATED MACROPHAGES

Treatment of recipient mice	No. of mice responding/total tested	Average haemagglutinin titre on day 7 ($1/\log_2$)
550 r, macrophages pre-incubated with SRBC	0/20	0
550 r, SRBC	1/28	0·07
Normal control, SRBC	28/28	10·4

volume of Hanks's solution to which an equal volume of cold distilled water was added with rapid shaking. Immediate reconstitution to isotonicity was obtained by adding 2 volumes of one and a half times concentrated Hanks's solution. The macrophages remained intact as attested by cytological smears made before and after such treatment. The 'ghosts' of the lysed SRBC were then washed from the macrophages by repeated gentle centrifugations. The washed macrophages were injected i.p. into irradiated recipients. In this preliminary experiment no group of irradiated mice, whether experimental or control, evinced a detectable anti-RBC response (Table 1).

Since the irradiation dose of 550 r was an arbitrary one based on previous studies with *Shigella* in C57BL mice (Gallily and Feldman, 1966, 1967), we proceeded to determine the threshold dose of irradiation above which CD_2 mice were unable to produce a detectable response to SRBC. In parallel, we did the same for the *Shigella paradysenteriae* antigen. As can

TABLE 2

THE EFFECT OF X-IRRADIATION DOSAGE ON THE RESPONSE OF CD_2 MALE MICE TO SRBC AND *Shigella*

X-ray dose (r)	No. of mice responding/No. of mice injected		Average titre* ($1/\log_2$)	
	SRBC	*Shigella*	SRBC	*Shigella*
250	14/15	20/20	10	10
350	20/28	18/19	3	8
400	4/8	NT	1	NT
450	4/18	16/20	0·3	3
500	1/8	NT	0·1	NT
550	0/18	4/11	0	0·8
650	NT	3/8	NT	0·8
Normal control	18/18	18/18	11	15

Mice immunized 48 hours after total-body irradiation and bled 6 days after immunization.

* Assays to detect anti-SRBC haemagglutinins and anti-*Shigella* agglutinins were performed as described in 'Materials and methods'. NT = Not tested.

be seen from Table 2, the minimum dose of irradiation which eradicated all but the slightest suggestion of a response to SRBC fell between 400 and 450 r, whereas to obliterate the anti-*Shigella* response, X-ray doses of 550 r or more had to be used. Experiments performed with *Shigella* antigen and an irradiation dose of 550 r confirmed the results of Gallily and Feldman (1966, 1967); sublethally irradiated mice, treated 48 hours after irradiation with normal peritoneal macrophages pre-incubated with *Shigella in vitro*, responded with anti-*Shigella* agglutinins (Table 3). Parallel experiments performed with whole SRBC and recipient mice irradiated with 400 or 450 r elicited totally negative results. These results

TABLE 3

RESPONSE OF SUBLETHALLY IRRADIATED CD_2 MICE TO *Shigella* OR SRBC FOLLOWING INOCULATION WITH PRE-INCUBATED MACROPHAGES

Treatment of recipient mice	Response on day 6			Response on day 8		
	No. of mice respond/ total tested	Average titre ($1/\log_2$)		No. of mice respond/ total tested	Average titre ($1/\log_2$)	
		Haem-agglutinin	anti-*Shigella* agglutinin		Haem-agglutinin	anti-*Shigella* agglutinin
550 r, macrophages pre-incubated with *Shigella*	45/45	—	7·1	40/41	—	7·8
550 r, *Shigella*	10/47	—	0·8	18/39	—	2·7
Normal control, *Shigella*	58/58	—	13	68/68	—	13
450 r, macrophages pre-incubated with SRBC	1/8	0·25	—	NT	NT	—
450 r, SRBC	3/8	0·3	—	NT	NT	—
400 r, macrophages pre-incubated with SRBC	1/16	0·06	—	1/16	0·06	—
400 r, SRBC	2/11	0·3	—	1/5	0·6	—
Normal control, SRBC	7/7	10	—	8/8	10	—

NT = Not tested.

led us to question whether the hypotonic treatment of the macrophage–SRBC suspension might be physiologically damaging to the macrophages even though no morphological changes were detected. Therefore, to eliminate the potentially detrimental step of haemolysis for the removal of the SRBC antigen, an immunogenically equivalent dose of RBC 'ghosts' in isotonic solution was substituted for whole SRBC in the incubation mixture. 'Ghosts', substituted in our protocol using 400 r irradiation, did not ameliorate our results (cf. Table 5).

Throughout these experiments, cytological smears of the macrophage suspensions were examined for phagocytosis of RBC. Depending upon the experiment, 0–5 per cent of the macrophages incubated with RBC in Hanks's solution exhibited phagocytosis. We, therefore, thought that the application of specific antibodies to SRBC to the *in vitro* incubation medium might improve phagocytosis (Feldman and Gallily, 1968) and concomitantly, perhaps, the processing of antigen for the immune response. In Table 4 are listed the concentrations of normal sera and antisera in Hanks's solution tested for their effect on the *in vitro* phagocytosis of SRBC by peritoneal macrophages. On the basis of the enhancement of phagocytosis, 20 per cent C57BL normal serum, and 1 per cent 5- and 11-day antisera were chosen as media for the incubation of macrophages with 'ghosts'. After incubation

TABLE 4

THE EFFECT OF VARIOUS NORMAL SERA AND ANTISERA ON THE *in vitro* PHAGOCYTOSIS OF SRBC BY PERITONEAL MACROPHAGES

Macrophages plus SRBC incubated with:	Total No. of macrophages counted	Phagocytosis(per cent)*
Hanks's medium	2100	2·4
20 per cent CD_2 normal serum	500	1·4
20 per cent C57BL normal serum	1700	5·6
2 per cent 5-day antiserum	400	68·5
1 per cent 5-day antiserum	800	93
0·2 per cent 5-day antiserum	966	34
2 per cent 11-day antiserum	1049	86
1 per cent 11-day antiserum	1200	96
0·5 per cent 11-day antiserum	926	76

All serum dilutions were made in Hanks's balanced salt solution.

* Per cent phagocytosis $= \frac{\text{Number of macrophages containing RBC}}{\text{Total number of macrophages}} \times 100.$

the macrophages were washed thoroughly and injected into mice exposed to 400 r 48 hours previously. The results of such experiments are tabulated in Table 5. None of the irradiated groups were capable of an immune response to SRBC under these experimental conditions.

If the macrophage were indeed the intermediary cell in the immune response to erythrocytes which was damaged by the sublethal irradiation, one ought to be able to reconstitute the immune response of such irradiated mice by a simultaneous injection of macrophages plus antigen. Such a procedure might ensure a completely physiological incubation of the macrophages with antigen in the peritoneal fluid of the recipient mouse. Table 6 demonstrates the ability of such a simultaneous injection to reconstitute the response to *Shigella* but not to SRBC.

It is a well-established fact that bacterial endotoxins function as adjuvants of the immune response (Braun and Nakano, 1965) and afford some level of protection against irradiation (Smith, Alderman and Gillespie, 1957). In an attempt to solve the enigma of

TABLE 5

THE RESPONSE OF SUBLETHALLY IRRADIATED CD_2 MICE TO SRBC FOLLOWING INOCULATION WITH MACROPHAGES PRE-INCUBATED WITH SRBC 'GHOSTS' AND VARIOUS NORMAL SERA AND ANTISERA

Treatment of recipient mice:	Response on day 6		Response on day 8	
	No. of mice responding/ total tested	average haemagglutinin titre ($1/\log_2$)	No. of mice responding/ total tested	average haemagglutinin titre ($1/\log_2$)
400 r, macrophages pre-incubated with 'ghosts' in Hanks's	0/20	0	1/20	0·1
400 r, macrophages pre-incubated with 'ghosts' in 20 per cent C57BL normal serum	0/15	0	6/15	1·2
400 r, macrophages pre-incubated with 'ghosts' in 1 per cent 5-day antiserum	3/10	0·5	1/15	0·2
400 r, macrophages pre-incubated with 'ghosts' in 1 per cent 11-day antiserum	4/15	0·6	3/15	0·6
400 r, 'ghosts' alone	15/34	1·2	9/30	1·1
Normal control, 'ghosts'	50/50	10	40/40	11

TABLE 6

THE RESPONSE OF SUBLETHALLY IRRADIATED MICE TO A SIMULTANEOUS INJECTION OF NORMAL PERITONEAL MACROPHAGES AND ANTIGEN

Treatment of recipient mice:	Response on day 6			Response on day 8		
	No. mice respond/ No. mice tested	Average titre ($1/\log_2$) Haemaggl.	*Shig.* aggl.	No. mice respond/ No. mice tested	Average titre ($1/\log_2$) Haemaggl.	*Shig.* aggl.
400 r, macrophages plus 'ghosts' or SRBC	4/17	0·8	—	2/10	0·4	—
400 r, 'ghosts' or SRBC	19/42	1·2	—	9/30	1·1	—
Normal control, 'ghosts' or SRBC	51/51	10	—	40/40	11	—
550 r, macrophages plus *Shigella*	20/20	—	8·5	19/19	—	11·7
550 r, *Shigella*	10/47	—	0·8	18/39	—	2·7
Normal control, *Shigella*	58/58	—	13	45/45	—	13

the different radiosensitive behaviour of the anti-*Shigella* and anti-RBC responses, experiments were performed to determine whether the endotoxin in the *Shigella* preparation might function non-specifically to enhance the immune response in sublethally irradiated mice. Mice irradiated with 550 r were inoculated with well washed samples of macrophages which had been incubated with 'ghosts' plus *Shigella*. As is evident from Table 7, anti-*Shigella* agglutinins were observed in the sera of mice recipients of macrophages pre-incubated with *Shigella* plus 'ghosts', whereas no response to SRBC was detectable.

TABLE 7

THE IMMUNE RESPONSE OF SUBLETHALLY IRRADIATED MICE TREATED WITH MACROPHAGES SIMULTANEOUSLY INCUBATED WITH *Shigella* AND SRBC 'GHOSTS'

Treatment of recipient mice:	Response on day 6			Response on day 8		
	No. of mice	Average titre ($1/\log_2$)* Haemaggl.	*Shigella* aggl.	No. of mice	Average titre ($1/\log_2$)* Haemaggl.	*Shigella* aggl.
550 r, macrophages preincubated with 'ghosts' and *Shigella*	15	0·07 (7)	6·5 (100)	15	0·5 (20)	8·1 (100)
550 r, 'ghosts'	16	0·1 (6)	—	15	0·3 (13)	—
550 r, *Shigella*	15	—	0·6 (20)	15	—	0·9 (27)
550 r	5	0 (0)	0·1 (20)	5	0 (0)	0·6 (20)
Normal control, 'ghosts' and *Shigella*	5	10 (100)	10 (100)	5	10 (100)	13 (100)

* The figures in parentheses represent the percentage of mice tested which evinced a positive agglutinin response.

DISCUSSION

The role of the macrophage as an intermediary in the development of an immune response to phage (Fishman, 1961; Fishman and Adler, 1963; Adler *et al.*, 1966; Gottlieb

et al., 1967), protein (Askonas and Rhodes, 1965; Pribnow and Silverman, 1967) and bacterial (Gallily and Feldman, 1966, 1967) antigens has been demonstrated. Recently, Mosier (1967) has described such a role for the macrophage in the response to SRBC *in vitro*. In contrast to the report of Mosier (1967), Perkins and Makinodan (1965) and Franzl and Morello (1966) found macrophages inhibitory to the anti-SRBC response.

In the experiments reported here, significant differences between the anti-*Shigella* and the anti-SRBC responses in sublethally irradiated mice were observed. Both the difference in irradiation dose needed to inactivate the immune response and the inability of an inoculum of macrophages plus antigen *in vivo* to reconstitute the anti-RBC activity suggested that the two immune responses functioned by different mechanisms. The results obtained could not be attributed to differences in sensitivity of assay since this should not alter the relative titres obtained between reconstituted sublethally irradiated mice and normal controls. In the experiments reported here the average *Shigella* agglutinin titre in macrophage-reconstituted, sublethally irradiated mice was lower than the anti-*Shigella* response of normal unirradiated mice by 1·3–5·9 $\log_2$ (see Tables 3, 6 and 7). Haemagglutinin titres elicited by sublethally irradiated, macrophage-treated mice were virtually undetectable and differed from those elicited by the normal controls by 10 $\log_2$ or more (see Tables 5, 6 and 7). The differences in the response to *Shigella* antigen and SRBC could not be attributed to a requirement for a larger pool of immune competent cells to elicit a detectable anti-SRBC response, since lowering of the irradiation to a threshold dose should have effectively overcome such a difficulty. From the studies reported by Perkins, Robinson and Makinodan (1961), it may be concluded that the minimum number of immune competent cells effective in eliciting a detectable anti-erythrocyte response in the mouse was smaller than that required for a demonstrable anti-*Shigella* response.

However, recent experiments in our laboratory have indicated that organ cultures of spleens from X-irradiated donors inoculated with macrophages and *Shigella in vitro* evoked an antibody response when normal thymus explants were added to the organ culture (Globerson and Feldman, unpublished). It, therefore, appears that normal macrophages triggered the formation of anti-*Shigella* antibodies by lymphoid cells which, following irradiation, had regenerated and achieved immune competence due to the inductive effect of the thymus. The regeneration of immune competent lymphocytes directed against various antigens may take place at different time intervals following irradiation. Such a stepwise development of immune competence has been described by Silverstein, Uhr, Kraner and Lukes (1963) during normal ontogeny of adaptive immunity in the foetal lamb. The post-irradiation time interval required for the regeneration of the capacity to respond to the SRBC may be longer than that for *Shigella*. If this is the case, our experiments may not have been performed at an optimal time to detect the possible response of lymphocytes to macrophages primed with SRBC. The possibility still exists that a second cell type, involved in the anti-SRBC response but not in the anti-*Shigella* response, is more radiosensitive than the macrophage (ref. Table 2). This is particularly feasible in view of the recent demonstration by Mitchell and Miller (1968) suggesting that two types of lymphocytes may be involved in the production of haemolysins to SRBC: the antigen-sensitive cells which are thymus-derived and the antibody-producing cells derived from bone marrow. Antibodies to sheep red blood cells are produced following the interaction between these two cell types. In fact, macrophages may not be involved at all in the production of anti-SRBC antibodies and the macrophage-like cells described by Mosier (1967) may simply represent the antigen-sensitive cells of Mitchell and Miller (1968). It

may then be assumed that these are radiosensitive cells which are lacking in the experimental system described in the present paper.

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REFERENCES

ADLER, F. L., FISHMAN, M. and DRAY, S. (1966). 'Antibody formation initiated *in vitro*. III. Antibody formation and allotypic specificity directed by ribonucleic acid from peritoneal exudate cells.' *J. Immunol.*, **97**, 554.

ASKONAS, B. A. and RHODES, J. M. (1965). 'Immunogenicity of antigen-containing RNA preparations from macrophages.' *Nature (Lond.)*, **205**, 470.

BRAUN, W. and NAKANO, M. (1965). 'Influence of oligodeoxyribonucleotides on early events in antibody formation.' *Proc. Soc. exp. Biol. (N.Y.)*, **119**, 701.

DRESSER, D. W. and WORTIS, H. H. (1965). 'Use of antiglobulin serum to detect cells producing antibody with low haemolytic efficiency.' *Nature (Lond.)*, **208**, 859.

FELDMAN, M. and GALLILY, R. (1968). 'Cell interactions in the induction of antibody formation.' *Cold Spring Harb. Symp. quant. Biol.*, **32**, 415.

FISHMAN, M. (1961). 'Antibody formation *in vitro*'. *J. exp. Med.*, **114**, 837.

FISHMAN, M. and ADLER, F. L. (1963). 'Antibody formation *in vitro*.' *Immunopathology*, 3rd Symposium, p. 79.

FRANZL, R. E. and MORELLO, J. A. (1966). 'The effect of peritoneal phagocytes on antibody production.' *J. Reticulo-endothelial. Soc.*, **3**, 351.

GALLILY, R. and FELDMAN, M. (1966). 'The induction of antibody production in X-irradiated animals by macrophages that interacted with antigen'. *Israel J. med. Sci.*, **2**, 358.

GALLILY, R. and FELDMAN, M. (1967). 'The role of macrophages in the induction of antibody in X-irradiated animals.' *Immunology*, **12**, 197.

GOTTLIEB, A. A., GLISEN, V. R. and DOTY, P. (1967). 'Studies on macrophage RNA involved in antibody production.' *Proc. nat. Acad. Sci. (Wash.)*, **57**, 1849.

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., NORDIN, H. and HENRY, C. (1963). 'The agar plaque technique for recognizing antibody-producing cells.' *Conference on Cell Bound Antibodies*, p. 109. Wistar Institute Press, Philadelphia.

KABAT, E. A. and MAYER, M. M. (1961). *Experimental Immunochemistry*, p. 149. Thomas, Springfield, Illinois.

MITCHELL, G. F. and MILLER, J. F. A. P. (1968). 'Immunological activity of thymus and thoracic-duct lymphocytes.' *Proc. nat. Acad. Sci. (Wash.)*, **59**, 296,

MOSIER, D. E. (1967). 'A requirement for two cell types for antibody formation *in vitro*.' *Science*, **158**, 1573.

PERKINS, E. H. and MAKINODAN, T. (1965). 'The suppressive role of mouse peritoneal phagocytes in agglutinin response.' *J. Immunol.*, **94**, 756.

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

PRIBNOW, J. F. and SILVERMAN, M. S. (1967). 'Studies on the radiosensitive phase of the primary antibody response in rabbits. I. The role of the macrophage.' *J. Immunol.*, **98**, 225.

SILVERSTEIN, A. M., UHR, J. W., KRANER, K. L. and LUKES, R. J. (1963). 'Fetal response to antigenic stimulus. II. Antibody production by the fetal lamb.' *J. exp. Med.*, **117**, 799.

SMITH, W. W., ALDERMAN, I. M. and GILLESPIE, R. E. (1957). 'Increased survival in irradiated animals treated with bacterial endotoxins.' *Amer. J. Physiol.*, **191**, 124.

STERZL, J. and RIHA, I. (1965). 'Detection of cells producing 7S antibodies by the plaque technique.' *Nature (Lond.)*, **208**, 858.

WEILER, E., MELLETZ, E. W. and BREUNINGER-PECK, E. (1965). 'Facilitation of immune hemolysis by an interaction between red cell-sensitizing antibody and γ-globulin allotype antibody.' *Proc. nat. Acad. Sci. (Wash.)*, **54**, 1310.