LYMPHOID CELLS IN DELAYED HYPERSENSITIVITY

III. The Influence of X-Irradiation on Passive Transfer and on In Vitro Production of Soluble Mediators*

By S. B. SALVIN and J. NISHIO

(From the Department of Microbiology, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania 15213)

(Received for publication 17 November 1971)

Delayed hypersensitivity is associated primarily with two types of cells, lymphocytes and monocytes. The lymphocytes become sensitized and ultimately appear in small numbers in sensitized animals at the site of intradermal injection of antigen (1); they also seem to be responsible for the adoptive transfer of delayed hypersensitivity from sensitized donors to normal recipients (2–4). Lymphocytes may also be active in the production in vitro of soluble mediators that influence the activity of macrophages. Herein, lymphocytes from sensitized guinea pigs are stimulated in vitro by specific antigen to produce one or more of these factors. Included among the activities of mediators that affect macrophages are (*a*) inhibition of migration (5), (*b*) aggregation (6, 7), (*c*) chemotaxis (8), (*d*) intracytoplasmic-granule formation (9), (*e*) interferon-like effects (10), and (*f*) lysis (11).

Although lymphocytes may be divided on the basis of such characteristics as size or duration of activity, they also seem to differ in their roles in delayed hypersensitivity (4). For example, sensitized lymphocytes from a variety of sources or tissues, such as spleen, lymph nodes, peritoneal exudate, peripheral blood, or bone marrow, all seem capable of producing soluble pharmacologic mediators in the presence of specific antigen. Nevertheless, lymphocytes from only a limited number of tissues are capable of transferring delayed hypersensitivity adoptively to normal recipients. Thus far, lymphocytes from the thymus or bone marrow of sensitized guinea pigs have not been demonstrated as having the capability of such adoptive transfers, although lymphocytes from such secondary lymphoid tissues as spleen and lymph nodes have this characteristic.

Total body X-irradiation of guinea pigs in doses greater than about 200 R greatly depletes the number of lymphocytes. Immunologically, this depletion becomes detectable when the X-irradiation is delivered 18–24 hr before the animal is challenged with an antigen. Subsequent formation of circulating antibody in response to this antigen may be markedly reduced (12). The expression of delayed hypersensitivity appears not to be materially affected (13). Guinea pigs exposed to 300 R total body irradiation and then inoculated with antigen in the footpads developed delayed

* This investigation was supported by the United States–Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (AI-08528).

hypersensitivity, as indicated by intradermal testing and adoptive transfer. This appearance of delayed hypersensitivity occurred, although the number of circulating eukocytes dropped from about 6000 to 400–600/mm^3 and the percentage of lymphocytes in the circulation increased from about 40% to 90–95%.

Since lymphocytes in the spleen and lymph nodes differed in their behavior from those in the thymus and bone marrow, the possibility was present that this difference might be expressed in the responses to immunosuppressive agents. Accordingly, experiments were initiated to determine what effect irradiation of whole animals or of lymphocyte suspensions would have on the expression of delayed hypersensitivity, as indicated by migration inhibition of normal macrophages in vitro on the one hand and by adoptive transfer to normal recipients on the other hand. In this paper, data are presented which show (*a*) that guinea pig lymphocytes irradiated in vivo or in vitro more readily lost their capacity to transfer delayed hypersensitivity adoptively than to produce soluble mediators in vitro, and (*b*) that adoptive transfer of delayed hypersensitivity is accompanied by a replication or maturation of certain donor cells.

Materials and Methods

Guinea Pigs.—When cells from individual animals were studied, Hartley guinea pigs weighing about 350–400 g each were used. When cells from several guinea pigs were pooled, inbred strain 13 guinea pigs were generally used. Adoptive transfer of delayed hypersensitivity was carried out with inbred strain 13 animals.

Guinea pigs were sensitized by inoculation into the four footpads of a total of 0.5 ml of Freund's adjuvant containing 2 mg of dried tubercle bacilli of the Jamaica strain. After an interval of 4 wk or more, the animals were skin tested with specific antigen, and the responses were read at 4 and 24 hr. At about this period, 20 ml of sterile, light mineral oil was introduced intraperitoneally, and 5–7 days later peritoneal exudate (PE)[1] cells were harvested. Other tissues were also removed for assay at this time, so that the capacity of lymphocytes from various tissues of the same donors could be simultaneously assayed in vitro for the production of soluble mediators and in vivo for the transfer of delayed hypersensitivity.

In the procedure for harvesting PE cells, a slit was made in the peritoneal wall of a guinea pig, and the peritoneal cavity was washed with 200 ml of sterile Hanks' solution. The suspension was washed three times with Hanks' solution by centrifugation for 10 min at 800–1000 rpm. The cells were then transferred to a 12 ml conical centrifuge tube and centrifuged for 10 min at 1200 rpm; the Hanks' solution was poured off and the tubes were inverted to drain off the excess moisture.

A supportive medium was prepared with 80% Hanks' solution and 20% normal inactivated guinea pig serum in 0.5% Ionagar (Colab Lab, Chicago Heights, Ill.). 100 units/ml of penicillin and 100 μg/ml of streptomycin were included. This agar was dispensed in Falcon plastic tissue culture flasks (3 ml/30 ml flask; Falcon Plastics, Div. B-D Laboratories, Inc., Los Angeles), or in Sykes-Moore chambers (Bellco Glass Inc., Vineland, N. J.). Antigen (e.g. 30 μg/ml of purified protein derivative [PPD]) was included, when necessary, in the agar. The packed cells were drawn up in a Pasteur pipette, and a small drop was subsequently

[1] *Abbreviations used in this paper:* LN, lymph node; MEM, minimal essential medium; MIF, migration-inhibitory factor; PE, peritoneal exudate; PPD, purified protein derivative; SP, spleen; T lymphocyte, thymus-derived cell.

carefully placed on the agar surface. The flasks were inverted and incubated at 37°C for 40–48 hr before the distance of cell migration was measured with the aid of an ocular micrometer in a dissecting microscope. The cells in the Sykes-Moore chambers were observed at irregular intervals for 24–48 hr for changes in morphology or cytology. The percentage of migration inhibition was calculated by subtracting from 1.0 the distance of migration of sensitized cells on the agar medium with antigen divided by the distance of migration of sensitized cells on the agar medium without antigen. The resulting figure was then multiplied by 100. Cells from normal animals were also studied on agar medium with and without antigen.

Guinea pigs or the lymphoid cells therefrom were irradiated in a ^{137}cesium unit, 0.662 Mev, producing 60 R/min at a distance of 35 cm from the source.

RESULTS

Induction of Delayed Hypersensitivity in Irradiated Recipients.—Experiments were initiated to determine whether lymphoid cells from hypersensitive donors could adoptively transfer delayed hypersensitivity to irradiated recipients. Normal guinea pigs were exposed to 900 R whole body radiation. At time intervals from day 1 to day 6 thereafter, quantities of lymphoid cells from donors sensitized to the tubercle bacillus were injected intravenously into the irradiated recipients, and these animals were skin tested immediately with 20 μg of PPD. Also, 2–4 $\times$ 10^8 bone marrow cells from normal guinea pigs were injected intravenously on the same day into irradiated recipients. Animals inoculated 1–6 days postirradiation with 2–4 $\times$ 10^8 lymph node cells from sensitized donors elicited skin reactions of 15–20 mm in diameter of induration (Table I). Guinea pigs similarly sensitized adoptively with 2–4 $\times$ 10^8 splenic cells developed slightly less induration after intradermal testing, i.e., 12–20 mm in diameter.

TABLE I

Response of Strain 13 Guinea Pigs, Irradiated with 900 R and Reconstituted with 2–4 $\times$ 10^8 Normal Bone Marrow Cells, to Lymphoid Cells from Donors Sensitized to 2 mg Tubercle Bacilli in Freund's Adjuvant

No. of guinea pigs (recipients)	Source of lymphoid cell	No. of donor cells ($\times$ 10^8)	Size of skin reaction (diameter of induration)
			mm
6	Lymph node	1.6–4	17–20
2	Lymph node + thymus	2.5–3.0 0.25	15–20
6	Spleen	2.8–4	12–20
1	Spleen + thymus	4 0.25	13
3	Bone marrow	8	0
2	Bone marrow + thymus	8 0.25	0
3	Control/spleen	3–6	13–20

In contrast, guinea pigs inoculated with as much as 8 $\times$ 10^8 bone marrow cells from sensitized donors did not show any indication of delayed hyper-

sensitivity after intradermal testing. Nor did irradiated recipients that had received combinations of 8×10^8 bone marrow cells and 3×10^7 thymus cells from sensitized guinea pigs indicate any hypersensitivity by skin test. Furthermore, the activity of lymph node or splenic cells was not enhanced by the addition of thymus cells. Splenic and lymph node cells from sensitized inbred strain 13 guinea pigs thus could induce delayed hypersensitivity, as measured by skin test, in irradiated recipients reconstituted with normal bone marrow cells.

Similar experiments were carried out with adoptive transfer of splenic, lymph node, or bone marrow cells into recipients that had not been reconstituted with normal bone marrow cells. The results were the same as with reconstituted recipients, in that splenic and lymph node cells, but not bone marrow cells, from sensitized donors could transfer delayed hypersensitivity.

Influence of X-Irradiation on Isolated Cells In Vitro.—Lymphoid cells from the spleen and lymph nodes of sensitized guinea pigs have the capacity to transfer delayed hypersensitivity to normal recipients, as measured by skin testing, and to produce soluble mediators in vitro in the presence of specific antigen. The possibility is present that these two expressions of delayed hypersensitivity may be associated with lymphocytes of different capacities or of different maturity. Therefore, cells from spleens, peritoneal exudate, or lymph nodes were exposed in vitro to various dosages of X-ray, and their capacities to transfer delayed hypersensitivity and to produce soluble mediators in vitro were assayed.

Each animal was sacrificed by a blow on the head, and the spleen and lymph nodes (inguinal, popliteal, and axillary) were removed. The tissues were carefully teased with fine needles, and the suspensions passed through glass wool filters. Suspensions containing at least 5×10^7 cells/ml in minimal essential medium (MEM) were then exposed to doses of X-irradiation varying from 120 to 3000 R. The cells were assayed for their immunologic capacities within 1–3 hr.

Exposure of sensitized lymph node or splenic cells to X-irradiation up to 3000 R and their subsequent incubation in vitro with antigen and normal PE cells indicated that the capacity to produce soluble mediators associated with migration inhibition had not been impaired (Table II). The degree of migration inhibition did not seem to be reduced perceptibly within the range of X-ray doses tested. When such irradiated cells, however, were injected intravenously into normal recipients of the same inbred strains, and the recipients were skin tested immediately, a definite effect of X-irradiation was observable on the capacity of lymphoid cells to transfer delayed hypersensitivity adoptively. After exposure of lymphoid cells to X-irradiation in doses up to 480 R, and injection into normal recipients, intradermal testing revealed little or no change in the extent of erythema and induration from the controls, i.e., those recipients that had received equivalent amounts of nonirradiated cells from the same donors. Splenic and lymph node cells were equally active.

When the amount of X-irradiation was increased to 600 R and above, the capacity to transfer delayed hypersensitivity adoptively to normal recipients was markedly impaired (Table II). At the level of 900 R, not one of eight recipients receiving 2×10^8 syngeneic irradiated lymph node or spleen cells indicated transfer of hypersensitivity by skin test. Thus, the phenomena associated with the adoptive transfer of delayed hypersensitivity in strain 13

TABLE II

Response to X-Irradiation of Splenic and Lymph Node Cells in Suspensions of 1–2 $\times$ 10^8/ml in MEM

Dose of X-ray	Percentage migration inhibition		Adoptive transfer no. animals positive /no. of animals tested		
	Sensitized LN cells + normal PE cells	Sensitized spleen cells + normal PE cells	With lymph node cells	With spleen cells	Total
R					
120	29	57	3/3	3/3	6/6
	13	42			
	23	40			
240	51	68	3/3	2/3	5/6
	31	27			
360	28	44	2/3	2/3	4/6
	15	63			
	38	58			
480	39	62	2/2	3/4	5/6
	25	14			
600	22	48	0/1	1/4	1/5
	—	41			
	46	24			
720	44	48	1/4	1/8	2/12
	38	27			
	38	36			
900	39	13	0/3	0/5	0/8
	29	26			
1500	29	19			
2400	16	35			
3000	18	38			

guinea pigs were more susceptible to X-irradiation than the phenomena associated with migration inhibition in vitro on exposure to specific antigen.

Influence of Time of Exposure to Irradiation.—The possibility occurs that cells irradiated in vivo are damaged, but are still effective in the expression of delayed hypersensitivity. Subsequent exposure of such cells to host reaction within the recipient's tissues may be the basis for the elimination of the capacity of lymphocytes to transfer delayed hypersensitivity adoptively. In one series of experiments, therefore, adoptive transfer and in vitro migration inhibition were examined the same day as irradiation. In another series of experiments, lymph

TABLE III

Response to Sensitized Donor Splenic, Lymph Node, and Peritoneal Exudate Cells to X-Irradiation Administered on Same Day as Adoptive Transfer or In Vitro Assay of Migration Inhibition

X-ray dosage	Percentage inhibition by			Adoptive transfer	
	PE	LN with normal PE cells	SP	No. of donor cells ($\times 10^8$)	Skin test (induration diameter)
R					*mm*
120		19*	35	8	18 × 17
					17 × 17
					15 × 17
240	47	32	30	8–9	13 × 16
					16 × 17
					18 × 19
					15 × 16
					10 × 12
360	34	35	29	5–8	18 × 17
					15 × 15
					12 × 12
					10 × 10
480	25	42	27	4–8	10 × 10
					<10
					<10
					<10
					10 × 11
					14 × 14
720	—	29	37	8	<10
					<10
					<10
1200	—	—	16	8	<10
					<10
					<10
Controls	42	27	30	6–11	22 × 22
					21 × 22
					18 × 20
					16 × 16
					17 × 17
					15 × 16
					15 × 15
					12 × 14
					12 × 13
					14 × 14
					11 × 11
					11 × 11

* These data are based on three separate experiments.

node and spleen cells were examined for their potential to produce migration inhibition in vitro only after remaining in the irradiated animal for an additional 24 hr after X-irradiation.

Strain 13 guinea pigs were exposed to total body X-irradiation, and the lymphoid cells were harvested. On the same afternoon normal recipients were inoculated intravenously with about 8×10^8 sensitized spleen, lymph node, or peritoneal exudate cells, and then were skin tested with 20 μg of PPD (Table III). Some lymphoid cells were simultaneously used for in vitro assay of migration inhibition of normal peritoneal exudate cells.

Adoptive transfer was successful after exposure of donors to total body irradiation up to 480 R. Above that dosage, transfer of delayed hypersensitivity could not be detected by skin test. In contrast, inhibition of migration was

TABLE IV

Skin Reactions in Normal Recipients of a Mixture of 6 × 10^8 Lymph Node, Spleen, and Peritoneal Exudate Cells

Time of skin testing after intravenous injection of sensitized cells							
0		24 hr		48 hr		72 hr	
4 hr	24 hr	4 hr	24 hr	4 hr	24 hr	4 hr	24 hr
—	15 × 15	—	28 × 30	7 × 7	16 × 15	14 × 14	17 × 17
—	8 × 8	—	27 × 28	10 × 12	15 × 15	8 × 9	14 × 16
0	13 × 13	—	30 × 30	13 × 12	19 × 18	18 × 19	18 × 19
0	12 × 13	0	8 × 8	0	16 × 16	12 × 13	10 × 12
		0	20 × 20	8 × 9	17 × 17	10 × 12	5 × 5
		0	18 × 17				
Positive + control	30 × 32	Control	0	Control	0	Control	0
Negative − control	0					Positive + control	30 × 34

detectable in doses up to 1200 R, the highest irradiation level studied (Table III).

Guinea pigs were irradiated with dosages up to and including 1200 R, but the lymphoid cells were not removed for in vitro assay until 24 hr later. Cells from the peritoneal exudate, spleen, and lymph node of sensitized animals all distinctly inhibited in vitro the migration of normal peritoneal exudate cells. Apparently, therefore, lymphoid cells which possibly had been damaged by irradiation were not eliminated by host phagocytes during the 24 hr post-irradiation.

Maturation or Replication of Donor Cells in Recipient Guinea Pigs.—After transfer of lymphoid cells from a sensitized donor to a normal recipient, a period involving maturation or replication of the donor cells may follow. X-irradiation of lymphoid cells may inhibit this replication or maturation of a given population of cells, without their actual destruction and elimination. If this were true, then insertion of a time interval after adoptive transfer of cells and before skin testing of a recipient should enhance the intradermal response.

Accordingly, 6×10^8 cells from the spleens, lymph nodes, or peritoneal exudates of sensitized strain 13 guinea pigs were injected intravenously into normal strain 13 guinea pigs, and different recipients were then skin tested at intervals from 0 to 72 hr thereafter (Table IV). Induration greater than 10×10 mm in diameter developed at the skin test site when antigen was injected intradermally immediately after adoptive transfer. The delayed responses, however, were greater when intradermal testing was done 24 or 48 hr after adoptive transfer. The delayed reactions were difficult to interpret when skin testing was done at 72 hr after cell transfer because of the appearance of Arthus-type responses. Thus, enhancement of the delayed response due to replication or maturation of cells seems to occur when sensitized lymphoid cells are transferred intravenously to normal recipients. Similar experiments were done with X-irradiated donors, and similar results were obtained.

Influence of Colchicine on Adoptive Transfer.—Since multiplication of cells in recipients seemed a probability, experiments were conducted to determine the influence of colchicine on adoptive transfer. 450–500 μg of colchicine/kg of guinea pig were injected intravenously into the recipients 48 hr before adoptive transfer, or the donor cells were mixed with that amount of colchicine before transfer. Both colchicine-treated and control animals were skin tested immediately after transfer.

TABLE V

Intradermal Reactions of Strain 13 Guinea Pigs Sensitized Adoptively to Tubercle Bacillus with about 8×10^8 Cells, Treated with 400-500 μg Colchicine/kg Body wt, and Skin Tested with 20 μg PPD

Type of donor cell	R_x* with colchicine	Control (no R_x with colchicine)
Lymph node	1/6‡	4/4
Spleen	0/8	3/3

* R_x = treated.

‡ No. of animals reacting/no. of animals tested.

A definite inhibition of intradermal response appeared in the recipients (Table V). The reduction in induration and erythema was most striking at 20 hr after intradermal testing; by 44–48 hr, however, the skin-test sites of colchicine-treated animals were almost as large as those in the controls, presumably because the effect of the colchicine in the inhibition of cell multiplication was temporary. When sensitized donor animals were similarly treated with colchicine 24–48 hr before adoptive transfer, the recipients did not indicate definite signs of inhibition of the skin response.

DISCUSSION

Delayed hypersensitivity is generally defined as the capacity of a sensitized animal to develop erythema and induration at the site of intradermal injection

of specific antigen. This induration is associated with the infiltration of mononuclear cells. In addition, delayed hypersensitivity is characterized by (*a*) adoptive transfer with lymphoid cells from a sensitized donor to a normal recipient (2), (*b*) in vitro production of mediators by lymphoid cells in the presence of specific antigen (14), and (*c*) association of sensitized lymphoid cells with enhanced cellular resistance in the intact animal (15). Whether all these manifestations of delayed hypersensitivity are associated with the same type of lymphocyte, however, is not known.

Cells from the spleen and lymph node of sensitized guinea pigs were found to have the capacity to transfer delayed hypersensitivity and to inhibit migration of normal peritoneal macrophages. Bone marrow and thymus cells, individually or in combination, from the same donors, were, however, not able to produce detectable delayed skin reactions adoptively in normal recipients of the same strain. The same cells were able in vitro to inhibit migration of normal macrophages (4). This difference in activity could be due to differences in quantity or quality of active cells in the various tissues. Increase in numbers of transferred cells did not result in the induction of detectable hypersensitivity in the recipient animals (4). The adoptive transfer of delayed hypersensitivity seemed to be associated with lymphocytes qualitatively or quantitatively different from those involved in migration inhibition in vitro.

This possible difference in the basic mechanisms associated with adoptive transfer of delayed hypersensitivity and with in vitro migration inhibition with lymphoid cells from animals with delayed hypersensitivity was further emphasized in studies with X-irradiation. Herein, the phenomena involved in adoptive transfer were more susceptible to irradiation, in vitro and in vivo, than those phenomena associated with migration inhibition in vitro. Since the production of migration-inhibitory factor (MIF) occurs after exposure of the cells to X-irradiation, the interruption of adoptive transfer must be associated with a process that does not depend on MIF production per se. The resistance of the migration-inhibition process further implies that the involved lymphocytes do not have to multiply on stimulation with specific antigen to secrete the compound or compounds associated with migration inhibition. In contrast, the radiosensitivity of the cells involved in adoptive transfer suggests that cell replication or maturation may be involved. This cell division could be the primary factor, whereby an increase in cell number is necessary before delayed hypersensitivity can be demonstrated. Thus, the ability to produce adoptive transfer should increase with time after cell transfer, a relationship which does seem to occur. Another possible need for cell division may lie in the production of a guinea pig "transfer factor," which is liberated in association with the process of mitosis.

The production of delayed hypersensitivity in the guinea pig is presumably associated with a thymus-derived cell, a T lymphocyte. The thymus cell itself, the thymocyte, is not capable of transferring delayed hypersensitivity adoptively, at least in cell numbers that are active with other secondary lymphoid

tissues. This T lymphocyte is present in the spleen, lymph node, peritoneal exudate, and peripheral blood. The cell is susceptible to X-irradiation, in that doses greater than about 480 R inhibit adoptive transfer. This inhibition could be due to restrictions of cell replication within the recipient, lack of production of a derivative cell in adequate numbers, or failure to produce a soluble factor in the replication/maturation process. Whether the same T lymphocyte is responsible for production of soluble factors in vitro has not yet been determined.

The apparent inactivity of thymus and bone marrow cells, or mixtures thereof, in adoptive transfer brings the transfer of delayed hypersensitivity into contrast with the production of circulating antibody. The immunologic complementation between thymus and bone marrow cells in the production of antibody (16, 17) does not seem to function in the adoptive transfer of delayed hypersensitivity. Yet, thymus and bone marrow cells are believed necessary for the induction of delayed hypersensitivity (18, 19). Thus, although primary lymphoid cells may be necessary for induction, a more mature derivative may be necessary for transfer. Also, the initial induction of delayed hypersensitivity in guinea pigs is relatively radioresistant, in contrast to the induction of antibody and to the adoptive transfer of delayed hypersensitivity, but similar to the process involving in vitro migration inhibition.

Pertinent to the observation that thymus or bone marrow cells from sensitized animals do not transfer delayed hypersensitivity, as measured by skin test, to a normal recipient, is the question whether lymphoid cells from various donor tissues appear in the recipient at the site of intradermal injection of antigen (1). Guinea pigs were sensitized in the footpads with either or both dead tubercle bacilli and/or dead *Candida albicans* in Freund's adjuvant, and selected animals were inoculated with tritiated thymidine. Lymphoid tissues were transferred to normal recipients in such a manner that each recipient received a mixture of cells from donors sensitized with tubercle bacilli and *C. albicans*, only one of which carried the radioactive label. A set of recipients also received doubly labeled and sensitized cells.[2]

When such animals were skin tested with PPD and candidin, and when the tissues were examined for infiltrated cells with radioactivity, each site of antigen injection contained the same numbers of radioactive lymphoid cells. This number was the same, whether lymph nodes or spleen provided the donor cells. When bone marrow cells were injected, the same numbers of labeled cells, in some cases even more, appeared at the skin-test sites, although skin tests were negative. Infiltration of donor cells occurs at the skin-test sites regardless of the degree of reaction and the type of donor lymphoid tissue, i.e., spleen, lymph node, or bone marrow. The presence of lymphoid cells from a sensitized donor at a skin-test site does not necessarily infer that reactivity is present.

[2] Salvin, S. B., R. Neta, and R. Mark. Unpublished results.

The number of immunocompetent donor cells necessary to elicit skin reactivity is small in comparison to the total number of donor cells that can infiltrate the skin-test site. This small number of immunocompetent cells at the skin test site makes the absolute demonstration of replication or maturation technically difficult.

SUMMARY

X-irradiation up to 480 R does not inhibit either adoptive transfer of delayed hypersensitivity or production in vitro of soluble mediators, such as migration-inhibitory factor (MIF). Above that dosage and as high as 3000 R, adoptive transfer is inhibited, but production of MIF is not. An increase in skin response occurred when 24–48 hr were allowed to elapse after intravenous transfer and before skin testing. Treatment of recipients with colchicine at the time of adoptive transfer inhibited the development of a skin reaction to specific antigen.

The authors gratefully acknowledge the technical assistance of Miss Judith Trout.

REFERENCES

1. McCluskey, R. T., B. Benacerraf, and J. W. McCluskey. 1963. Studies on the specificity of the cellular infiltrate in delayed hypersensitivity reactions *J. Immunol.* **90**:466.
2. Chase, M. W. 1945. The cellular transfer of cutaneous hypersensitivity to tuberculin. *Proc. Soc. Exp. Biol. Med.* **59**:134.
3. Metaxas, M. N., and M. Metaxas-Buhler. 1955. Studies on the cellular transfer of tuberculin sensitivity in the guinea pig. *J. Immunol.* **75**:333.
4. Salvin, S. B., J. Nishio, and M. Gribik. 1970. Lymphoid cells in delayed hypersensitivity. I. *In vitro* vs. *in vivo* responses. *Cell. Immunol.* **1**:62.
5. Bloom, B. R., and B. Bennett. 1966. Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science (Washington).* **153**:80.
6. Salvin, S. B., J. Nishio, and M. S. Lin. 1969. Cell activity in delayed hypersensitivity *in vitro*. *Fed. Proc.* **28**:629. (Abstr.)
7. Lolekha, S., S. Dray, and S. P. Gotoff. 1970. Macrophage aggregation *in vitro*: a correlate of delayed hypersensitivity. *J. Immunol.* **104**:296.
8. Ward, P. A., H. G. Remold, and J. R. David. 1970. The production of antigen-stimulated lymphocytes of a leucotactic factor distinct from migration inhibitory factor. *Cell. Immunol.* **1**:162.
9. Salvin, S. B., and J. Nishio. 1969. *In vitro* cell reactions in delayed hypersensitivity. *J. Immunol.* **103**:138.
10. Subrahmanyan, T. P., and C. A. Mims. 1970. Interferon production by mouse peritoneal cells. *J. Reticuloendothel. Soc.* **7**:32.
11. Granger, G. A., S. J. Shacks, T. W. Williams, and W. P. Kolb. 1969. Lymphocyte *in vitro* cytotoxicity: specific release of lymphotoxin-like materials from tuberculin-sensitive lymphoid cells. *Nature (London).* **221**:1155.
12. Taliaferro, W. H., and L. G. Taliaferro. 1951. Effect of X-rays on immunity: a review. *J. Immunol.* **66**:181.

13. Salvin, S. B., and R. F. Smith. 1959. Delayed hypersensitivity in the development of circulating antibody. The effect of X-irradiation. *J. Exp. Med.* **109**:325.
14. Rich, A. R., and M. R. Lewis. 1932. The nature of allergy in tuberculosis as revealed by tissue culture studies. *Bull. Johns Hopkins Hosp.* **50**:115.
15. Mackaness, G. B. 1970. The monocyte in cellular immunity. *Seminars Hematol.* **7**: 172.
16. Claman, H. N., E. A. Chaperon, and R. F. Triplett. 1966. Immunocompetence of transferred thymus-bone marrow cell combinations. *J. Immunol.* **97**:828.
17. Claman, H. N., and E. A. Chaperon. 1969. Immunologic complementation between thymus and marrow cells—a model for the two-cell theory of immunocompetence. *Transplant. Rev.* **1**:92.
18. Lubaroff, D. M., and B. H. Waksman. 1967. Delayed hypersensitivity: bone marrow as the source of cells in delayed reactions. *Science (Washington).* **157**: 322.
19. Miller, J. F. A. P., and D. Osoba. 1967. Current concepts of the immunological function of the thymus. *Physiol. Rev.* **47**:437.