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3 August 1970

Radioresistance of Cooperative Function of Carrier-Specific Lymphocytes in Antihapten Antibody Responses

Abstract. *Transfer of lymphoid cells from strain-2 guinea pigs immunized to bovine gamma globulin into syngeneic recipients immunized with dinitrophenyl ovalbumin markedly enhances the secondary antidinitrophenyl response of the recipient to challenge with dinitrophenyl–bovine gamma globulin. This function of the carrier bovine gamma globulin–specific cells is resistant to irradiation with up to 5000 rads, although the capacity of the irradiated cell population to transfer immunologic memory for bovine gamma globulins or to be stimulated by antigen to synthesize DNA in vitro is abolished by this treatment.*

The production of hapten-specific antibodies in response to immunization with haptenic carrier conjugates can be markedly enhanced by preimmunization of animals with unmodified carrier. This can be demonstrated strikingly in secondary antihapten antibody responses to hapten conjugates of a protein not used in primary immunization (*1–3*). Furthermore, preimmunization with carrier enhances the primary antihapten antibody response to a hapten-carrier complex as well (*3*). These findings suggest the existence of independent recognition units for hapten and for carrier determinants, the cooperation of which is of considerable importance in the development of hapten-specific immune responses.

It has been amply demonstrated that the functional carrier recognition unit is not conventional humoral antibody (*2, 3*). In the adoptive secondary antibody response in mice, Mitchison and his collaborators have shown that cooperative interactions are mediated by hapten-specific and carrier-specific lymphoid cells (*2, 4*). More recently we have demonstrated that transfer of live lymphoid cells from strain-2 guinea pigs immunized with bovine gamma globulin (BGG) to syngeneic recipients previously primed with 2,4-dinitrophenyl-ovalbumin (DNP-OVA) enhances anti-DNP antibody responses of these recipients to subsequent challenge with DNP-BGG (*5*). Thus, in both species the carrier recognition unit appears to be associated with specific lymphoid cells. In this report we present data showing that the carrier function of these lymphoid cells is radioresistant.

The experiments were carried out in strain-2 guinea pigs. Donor guinea pigs were immunized in the four footpads with either 50 μg of BGG emulsified in complete Freund's adjuvant (CFA) or saline emulsified in CFA. Three weeks later the animals were killed and axillary, occipital, inguinal, and popliteal lymph nodes and spleen were removed. Single cell suspensions, in minimum essential medium (Eagle), were prepared and washed. In each experiment, cell suspensions from BGG cell donors and CFA cell donors were pooled separately. Portions of the pooled cells were either irradiated (*6*) or not irradiated. Portions not irradiated were nevertheless subjected to identical conditions of handling employed for cell portions receiving irradiation, such as length of time exposed to ambient temperature. Subsequent to all manipulations, cell viability was 70 percent for both irradiated and nonirradiated cell populations, as shown by trypan blue exclusion.

Varying numbers of these cells were injected intravenously into syngeneic recipients which had been immunized 3 weeks earlier with three daily doses of 1.0 mg of DNP_7-OVA (*7*) administered intraperitoneally in saline. Recipients were given a booster dose 6 days after cell transfer with 1.0 mg of DNP_{28}-BGG in saline (200 μg intradermally followed by 800 μg intraperitoneally 4 hours later) (*8*). Animals were bled just prior to secondary immunization (day 0) and 4, 7, and 11 days later. Serum anti-DNP antibody levels were determined in a modified Farr assay by using tritiated DNP-epsilon-aminocaproic acid (*3, 9*). Quantitative determination of precipitating anti-BGG antibody was performed with I^{125}-labeled BGG as previously described (*3, 10*).

Table 1 presents the results from two experiments in which recipients received 1.2 to 1.3 $\times$ 10^9 donor cells. In the first experiment, there was a marked enhancement of the anti-DNP responses in recipients which received either nonirradiated BGG cells or BGG cells irradiated with 1500 rads as compared with responses of recipients

Table 1. Effect of in vitro irradiation of carrier-specific cells on their capacity to enhance hapten-specific anamnestic responses. N.D., not determined.

Protocol*		Animals (No.)	Anti-DNP antibody (μg/ml)†				Anti-BGG antibody (P_{50})†		
Specificity of cells transferred	Irradiation (rads)		Day 0	Day 7	Day 11	"Boost"	Day 0	Day 7	"Boost"
			Experiment 1						
BGG	None	3	0.52	63.8	108.3	107.8	47.6	82.5	34.9
BGG	1500	4	1.0	110.7	264.6	263.6	<5	<5	0
CFA	None	5	0.82	0.66	16.8	16.0	<5	<5	0
			Experiment 2						
BGG	None	4	1.1	25.7	254.3	253.2	48.4	80.6	32.2
BGG	1500	4	1.2	12.4	148.4	147.2	<5	<5	0
BGG	5000	4	0.94	2.3	47.5	46.6	<5	<5	0
CFA	None	4	.58	0.34	8.3	7.7	N.D.	N.D.	

* 1.2 to 1.3 $\times$ 10^9 donor lymph node and spleen cells were transferred to each recipient which had been previously immunized with DNP_7-OVA. Recipients were given booster doses with DNP_{28}BGG 6 days after transfer. † The data are expressed as geometric means. "Boost" represents the increase in mean antibody levels from day 0 to day 11 for anti-DNP and from day 0 to day 7 for anti-BGG. A comparision of the geometric mean "boosts" in serum anti-DNP antibody concentrations, from day 0 to day 11, gave the following results (determined by Student's *t*-test): In experiment 1 comparison of the groups receiving nonirradiated and irradiated BGG cells with the group receiving CFA cells yielded *P* values of $.05 > P > .02$ and $P < .01$, respectively. In experiment 2, comparison of groups receiving nonirradiated, 1500 rads, and 5000 rads irradiated BGG cells with the group receiving CFA cells yielded *P* values of $P < .01$, $P > .01$, and $.02 > P > .01$, respectively.

Table 2. Effect of in vitro irradiation of carrier-specific cells on their capacity to enhance hapten-specific anamnestic responses.

Protocol*		Animals (No.)	Anti-DNP antibody (μg/ml)†			
Specificity of cells transferred	Irradiation (rads)		Day 0	Day 7	Day 11	"Boost"
BGG	None	10	1.0	12.8	62.0	61.0
BGG	1000	5	1.2	18.6	89.6	88.4
BGG	3000	5	0.65	30.8	91.6	90.9
BGG	5000	5	1.5	20.8	200.6	199.1
CFA	None	5	0.41	0.12	5.5	5.1
CFA	3000	5	.43	.16	3.6	3.2

* 400 to 500 $\times$ 10^6 donor lymph node and spleen cells were transferred to each recipient which had been previously immunized with DNP_7-OVA. Recipients were given booster doses with DNP_{28}-BGG 6 days after transfer. † The data are expressed as geometric means. "Boost" represents the increase in mean antibody levels from day 0 to day 11. A comparison of the geometric mean "boosts" from day 0 to day 11 of each group receiving BGG cells with those of the groups receiving CFA cells yielded a P value of $< .01$ in each case (determined by Student's t-test).

which received control CFA cells. Enhanced responses in this experiment were clearly evident by day 7 after challenge with DNP_{28}-BGG. In the second experiment, enhanced anti-DNP responses were manifested by the recipients of BGG cells despite irradiation with as much as 5000 rads. However, the degree of enhancement of anti-DNP antibody responses by BGG cells was diminished by irradiation with 5000 rads. Although the enhancing effect was evident by day 7 in recipients of BGG cells as compared with recipients of CFA cells, the magnitude of the enhancement phenomenon was more clearly seen at day 11 in this experiment. In both experiments, recipients of nonirradiated BGG cells had measurable circulating anti-BGG antibody prior to challenge with DNP_{28}-BGG and manifested secondary anti-BGG responses by day 7 after the booster dose. In contrast, recipients of irradiated BGG cells had no measurable circulating anti-BGG antibody prior to or after challenge with DNP_{28}-BGG, demonstrating the suppressive effect of the doses of irradiation employed on the cells which are subsequently responsible for the synthesis of anti-BGG antibody.

Table 2 presents the results from a third experiment in which recipients received 400 to 500 $\times$ 10^6 donor cells. Again, the enhanced anti-DNP responses of recipients which received BGG cells were clearly evident by day 7. Radiation doses of 1000, 3000, and 5000 rads had no diminutive effect, in contrast to the second experiment in Table 1. In fact, by day 11 the highest anti-DNP responses were manifested by recipients of BGG cells irradiated with 5000 rads. That the expression of enhanced antihapten responses does not result from a nonspecific effect caused by heavy irradiation of lymphoid cells is demonstrated by the absence of enhanced responses in recipients given CFA cells which had been irradiated with 3000 rads.

Finally, a study was made to determine the effects of in vitro irradiation on the capacity of carrier-specific cells to synthesize DNA in response to antigenic stimulation. Lymph node cell suspensions were prepared (*11*) from guinea pigs immunized 3 weeks previously with 50 μg of BGG in CFA. Portions were exposed to varying doses of irradiation (Table 3) and then cultured in triplicate in the presence or absence of antigen (100 μg of DNP_{43}-BGG per milliliter of medium). [^{3}H]Thymidine was added 24 hours later and the cultures were allowed to incubate for an additional 24 hours. The amount of [^{3}H]thymidine incorporated into DNA was measured and the percent inhibition of DNA synthesis at each dose of radiation employed was determined. As shown in Table 3, antigen-stimulated DNA synthesis is inhibited by 94 percent at a dose of 1500 rads and by more than 99 percent with 3000 rads and 5000 rads.

The precise mechanism or mechanisms of cooperative interaction between cells in the immune response to hapten-carrier conjugates is unknown.

Table 3. Effect of in vitro irradiation of carrier-primed lymph node cells on antigen-stimulated DNA synthesis.

Irradiation (rads)	Baseline CPM*	ΔCPM†	Inhibition of ΔCPM (%)
0	1,323	19,149	0
250	236	6,134	68
500	74	1,759	91
1,500	25	1,090	94
3,000	11	188	99.2
5,000	13	32	99.8

* Radioactivity incorporated into DNA by cells incubated in the absence of antigen. † Radioactivity incorporated into DNA in response to stimulation with 100 μg of DNP_{43}-BGG per milliliter of medium minus that incorporated in the absence of antigen.

The evidence accumulated thus far suggests several possibilities: (i) The carrier cell may serve to concentrate antigen and present it to the hapten-specific cells. (ii) The carrier cell may provide a signal of some sort which is crucial to the differentiation or proliferation, or both, of the hapten-specific cell once it has interacted with antigen. (iii) The carrier-specific cell may secrete a special class of anti-carrier antibody which must interact with antigen before appropriate stimulation of hapten-specific cells can occur. (iv) The carrier cell may be required to process the antigen before the latter can specifically stimulate hapten-specific cells.

There appears to be an analogy between carrier function in the antihapten antibody response to hapten-carrier immunizations and the requirement for thymus-dependent cells in the synthesis of antibody to sheep erthrocytes by bone marrow–derived cells in the mouse (*12*). Indeed, preliminary evidence of Mitchison *et al.* indicates that the lymphoid cells responsible for carrier function in mice are most likely thymus-derived (*4*). Claman and coworkers have found that in thymus-marrow synergism in mice both cell populations are sensitive to irradiation (*13*). Thus, even after a period of proliferation in the presence of antigen the capacity of thymus cells to interact synergistically with normal bone marrow cells was abrogated by irradiation. In contrast, Goldie and Osoba have reported synergism between heavily irradiated (up to 2500 rads) and nonirradiated normal mouse spleen or lymph node cells in the development of plaque-forming cells to sheep erythrocytes in vitro (*14*). Kennedy has made similar observations (*15*).

Our findings do not necessarily contradict the findings of Claman and coworkers cited above (*13*). The conditions of their experimental system appear to require a period of proliferation by thymus-derived cells in the presence of antigen before such cells can facilitate activity of normal bone marrow cells. In the present studies, proliferation of the responsible cells was not required or expected to occur in nonirradiated recipients.

The present studies provide evidence that the functional role of the cells which serve as the carrier recognition unit in hapten-carrier cooperation is quite resistant to irradiation. Thus, despite doses of irradiation as high as 5000 rads, carrier-primed lymphoid cells

were capable of promoting strikingly enhanced antihapten antibody responses to the specific hapten-carrier conjugate in syngeneic guinea pig recipients. This enhancement of the antihapten response occurred even though the capacity of the carrier-primed cells to subsequently synthesize and secrete anticarrier antibody in vivo or to synthesize DNA in response to antigenic stimulation in vitro was completely abrogated by the doses of irradiation employed. Furthermore, the facilitative capacity of irradiated cells was intact after the 6-day interval between the transfer of cells and the subsequent immunization. These findings indicate that (i) proliferation is not required by the carrier-specific cell to perform its functional role in cooperative interaction with the hapten-specific cell; and (ii) the heavily irradiated carrier cell is either capable of surviving and performing its specific function in vivo for at least as long as 6 days after irradiation and transfer, or it performs its role through interaction with other cells prior to subsequent immunization, and therefore its presence is not required at the precise time of antigenic stimulation.

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6. Irradiation of cells was carried out with a Westinghouse 250-kv (peak) machine at a rate of 674 rad/min, under the following conditions: 200 kv, peak at 15 ma, focal distance 25 cm.
7. DNP conjugates were prepared under alkaline conditions as described (*3*). Subscripts refer to the average number of moles of DNP per mole of protein.
8. The interval of 6 days between cell transfer and secondary challenge has been shown in a previous study (*5*) to be optimal in eliciting the enhanced secondary antihapten response in guinea pigs.
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11. Lymph node cell suspensions were prepared, under sterile conditions, in tissue culture medium, RPMI-1640 (Grand Island Biological Co.) supplemented with glutamine (2 μmole/ml) and penicillin (100 units/ml), streptomycin (25 μg/ml) and 10 percent guinea pig serum; 5×10^6 cells were cultured in a total volume of 1.5 ml per tube at 37°C in a humid atmosphere of 5 percent CO_2 in air; 1 μc of [^{3}H]thymidine was added to each culture tube.
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12 June 1970

Electrical Coupling: Low Resistance Junctions between Mitotic and Interphase Fibroblasts in Tissue Culture

Abstract. *Dividing cells of chick embryonic fibroblasts and of mouse embryonic fibroblasts (3T3) in tissue culture are electrically coupled to their interphase neighbors. Recordings from many such cells suggest that this coupling persists throughout the division cycle of the mitotic cell.*

The presence of low-resistance coupling between excitable as well as between nonexcitable cells in vivo is well documented (*1, 2*). Moreover, animal cells can establish and maintain low-resistance junctions when grown in tissue culture (*2, 3*). Although the function of the low-resistance junctions between nonexcitable cells is not known, the prevalence of these junctions between cells in vivo and in vitro suggests that they are basic to cell activity. We now present evidence demonstrating the existence of low-resistance coupling between normal interphase and mitotic fibroblasts in tissue culture.

The coupling measurements were made with (i) secondary chick embryonic fibroblasts prepared as described by Rein and Rubin (*4*) and grown on 50-mm plastic petri dishes in 5 ml of medium 199 containing 2 percent tryp-

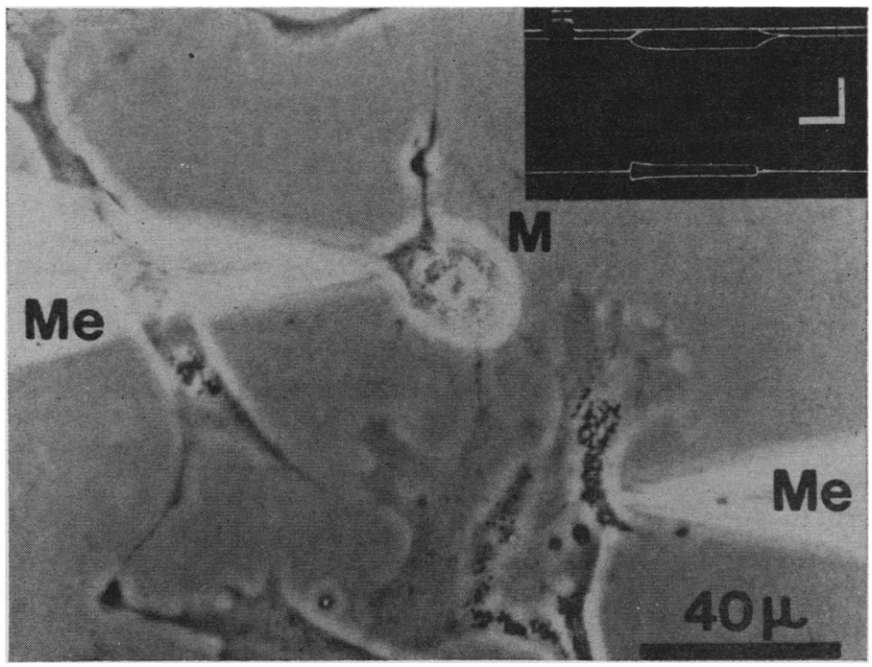

Fig. 1. Phase contrast picture of two normal fibroblasts after impalement with microelectrodes (*Me*). One cell (*M*) is in mitosis, and the other is in interphase (1 day in culture). (Inset) The electrical record indicates that the cells are functionally coupled. Current pulses (4×10^{-9} ampere and 56 msec) were passed in either direction through the cell membrane of the mitotic cell (*M*), and symmetrical voltage changes were recorded within the interphase cell. The rectangular pulses to the left in the top two traces (also inset of Fig. 3) are calibrating pulses used to monitor frequency and amplitude response. Positive calibrating pulse: 10 mv, 10 msec. Vertical white bar: 20 mv for top two electrical traces, 20×10^{-9} ampere for bottom current traces. Horizontal white bar: 20 msec. Inset traces were retouched slightly.