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T-Cell Regulation of T-Cell Responses to Antigen*

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Summary. Parental thymocytes, inoculated into F_1 mice which have been depleted of lymphoid cells by lethal irradiation, react to the host antigens which are contributed by the reciprocal parent in the F_1 cross, by synthesizing DNA. The amount of DNA the parental thymocytes synthesize can be regulated by the addition of F_1 thymocytes to the lethally irradiated F_1 recipient. F_1 thymocytes suppress the response of a highly responding inoculum of parental thymocytes and boost the response of an inoculum responding less well. These regulatory effects of F_1 thymocytes are not abolished by 900 R of irradiation. 900 R-irradiated parental cells which are themselves incapable of DNA synthesis can also affect the response of DNA synthesizing cells. As with F_1 thymocytes, the effect that 900 R-irradiated parental thymocytes has depends on the activity of the DNA synthesizing population; when it is high the effect is suppressive, when it is low there is augmentation. These results indicate that during the course of their response to antigen, T cells may transmit signals to other T cells which in turn transmit feedback signals to the primarily responding cells and in so doing alter their response. Such circular interactions between T cells may play an important role in immunological homeostasis.

INTRODUCTION

The addition of F_1 spleen cells to inocula of parental thymocytes in sublethally irradiated F_1 hosts has been shown to affect the splenomegaly that results from the ensuing graft-versus-host reaction (GVHR) (Hilgard, 1970; Barchilon, Liebhaber and Gershon, 1972; Liebhaber, Barchilon and Gershon, 1972). The effect the F_1 spleen has depends, to a large degree, on the dose of parental thymocytes used to initiate the GVHR; at high parental cell doses F_1 cells increase spleen size while at lower doses of parental cells, they decrease it (Barchilon, Liebhaber and Gershon, 1972; Liebhaber, Barchilon and Gershon, 1972). Our analysis of the mechanism by which the F_1 cells produce these bidirectional effects was hampered somewhat by the fact that the assay used for measuring parental cell activity required proliferation of the F_1 cells. The splenomegaly produced in GVHR is due to the proliferation of F_1 cells (Biggs and Payne, 1959; Davies and Doak, 1960; Fox, 1962; Zeiss and Fox, 1973; Nowell and Defendi, 1964; Owen, Moore and Harrison, 1965; Nisbet and Simonsen, 1967; Nakic, Kastelan, Mikuska and Bunarevic, 1967; Elkins, 1970). It was impossible therefore to determine how the two interacting cell populations were affecting each other.

We have developed a new assay for GVHR which measures the activity of the cells

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responding directly to the hosts antigens (Gershon and Hencin, 1971; Gershon and Liebhaber, 1972). This consists of injecting lethally irradiated F_1 mice with parental thymocytes and quantitating the amount of DNA the injected cells synthesize. Using this technique we have found that the addition of F_1 thymocytes to inocula of parental thymocytes in lethally irradiated F_1 recipients can significantly alter the DNA synthetic response of the parental cells (Gershon, Cohen, Hencin and Liebhaber, 1972; Gershon, Lance and Kondo, 1974). Bidirectional effects, similar to those noted to be produced by the F_1 spleen cells using the splenomegaly assay, can occur (Gershon, Lance and Kondo, 1974). In addition, analysis of kinetic studies of parental cells responding in the absence of F_1 cells indicates that interactions with bi-directional effects may occur between the parental cells as well as between them and host (F_1) cells (Gershon and Liebhaber, 1972; Cohen and Gershon, submitted for publication).

The concept of interacting subpopulations of T cells in GVHR is now well established (Asofsky, Cantor and Tigelaar, 1971; Cantor and Asofsky, 1970; Cantor and Asofsky, 1972; Tigelaar and Asofsky, 1972, 1973), although only positive interaction effects have been noted so far. However, it has been shown, using other assay systems, that removal of one of the interacting T-cell subpopulations, referred to as T_1 (Raff and Cantor, 1971), may result in increased immune responses of the T_1-deprived animals (Mosier and Cantor, 1971; Kerbel and Eidinger, 1972). The reasons for the discrepancies between these reports are not clear but it may be that there are indeed bidirectional interaction effects between the two T-cell populations and that different studies have maximized the conditions for producing one or the other effects.

In the present study we have examined the effect of two different cell populations on the DNA synthetic response of parental thymocytes in F_1 mice. We used F_1 thymocytes which do not synthesize DNA on their own in F_1 mice and also parental thymocytes rendered incapable of DNA synthesis by irradiation with 900 R. Both cell populations have the ability to augment or suppress the parental cell response. Which effect they will have depends largely on the activity of the parental cells responding alone; when it is high the non-responding cells act as suppressors, when it is low they act as boosters. Thus, their effect on the parental cells depends to a degree on the effect the parental cells have on them. This type of circular interaction may play an important role in immunological homeostasis.

MATERIALS AND METHODS

Production of GVH disease

GVHR was produced in the offspring of C_3H (H-2K) $\times$ DBA/2 (H-2D) matings (CDF_1) by the intravenous inoculation of C_3H thymocytes. Recipient mice were lethally irradiated on the day of thymocyte inoculation.

Mice

All of the mice were males from Jackson Laboratory, Bar Harbor, Maine. After receipt, they were rested 1 week in our colony before use. Recipients (CDF_1) were 7–8 weeks of age and thymocyte donors (C_3H and CDF_1) were 5 weeks old.

Irradiation

Recipient mice were placed in plexiglass chambers on a rotating platform and received

900 R of X-irradiation from a Siemens 250KV machine (Siemens Corporation, Iselin, New Jersey) with a 2 mm aluminum filter at a dose rate of 85 R/minute. Cells were irradiated in iced Petri dishes in the same fashion.

Cell suspensions

Thymus cell suspensions were made by gently squeezing thymuses between two sterile glass slides. The cells were then filtered through gauze and washed twice in cold, sterile medium 199 with 100 units/ml penicillin, streptomycin, kanamycin and 10 units/ml heparin. Viable cells were counted using the Trypan Blue dye exclusion method. Inoculations were made in a volume of 0·2 ml via the tail vein.

Measurement of DNA synthesis

Uptake of the thymidine analogue 5-iodo-2-deoxyuridine (IUDR) labelled with ^{125}I was used as a measure of DNA synthesis. It has been shown that 24 hours after its inoculation, all IUDR not intimately associated with DNA is cleared from the lymphoid tissue (Fox and Prusoff, 1965). Thus, test mice were injected intraperitoneally with 2 μCi of [^{125}I]IUDR in a 0·2 ml volume 2, 3, 4 and 5 days after GVH inception, and their spleens and femoral lymph nodes were harvested 24 hours later. The [^{125}I]IUDR used in these experiments came from three different sources, Amersham-Searle Corporation (Arlington Heights, Illinois), Schwarz Bio Research Incorporated (Orangeburg, N.Y.) and New England Nuclear Corporation (Boston, Massachusetts), and varied between 2 and 6 μCi/μg in specific activity. At the time of inoculation of the IUDR into the mice, 0·2 ml of the isotope was placed in a counting tube. This served as a standard and after counting for 6 minutes in a Nuclear-Chicago (Nuclear-Chicago Corporation, Des Plains, Illinois) gamma counter, the standard minus the background was divided into the results minus the background; the results are therefore expressed as percentage uptake of label.

Background DNA synthesis

Previous work has shown that lethally irradiated mice incorporate very low levels of [^{125}I]IUDR during the first 5 days after irradiation and that inoculation with syngeneic thymocytes does not significantly increase this level (Gershon and Hencin, 1971). Therefore, controls not inoculated with thymocytes or inoculated with syngeneic thymocytes were not routinely included in all experiments. Several of the experiments reported below did, however, include such controls. Also, since previous work has shown a return of DNA synthesis in the spleens of lethally irradiated mice on day 6 post-irradiation (Gershon and Hencin, 1971), we confined our meaurements to the first 5 days.

RESULTS

EFFECT OF PARENTAL THYMOCYTE DOSE ON THE REGULATORY EFFECTS OF A STANDARD DOSE OF F_1 THYMOCYTES

We inoculated different doses of parental thymocytes alone or mixed with a standard dose of F_1 cells into lethally irradiated F_1 mice and measured the subsequent DNA synthesis of the inoculated cells. We report the results of two consecutive experiments in which three doses of parental cells were used, x, 2/3x, and 4/9x. In these experiments we used the same number of parental thymocyte donors (thirty-five) for the same number of F_1 recipients (seventy-five). The yield of cells was extremely different in the two experiments.

We used all the cells we harvested in both experiments so there is a three-fold difference in x; $x = 9 \times 10^7$ in the first experiment and 3×10^7 in the second. We also used all the cells harvested from ten F_1 thymocyte donors; this worked out to $2{\cdot}5 \times 10^7$ cells per recipient in the first experiment and $7{\cdot}5 \times 10^6$ in the second. In spite of these differences the results of the two experiments are strikingly similar.

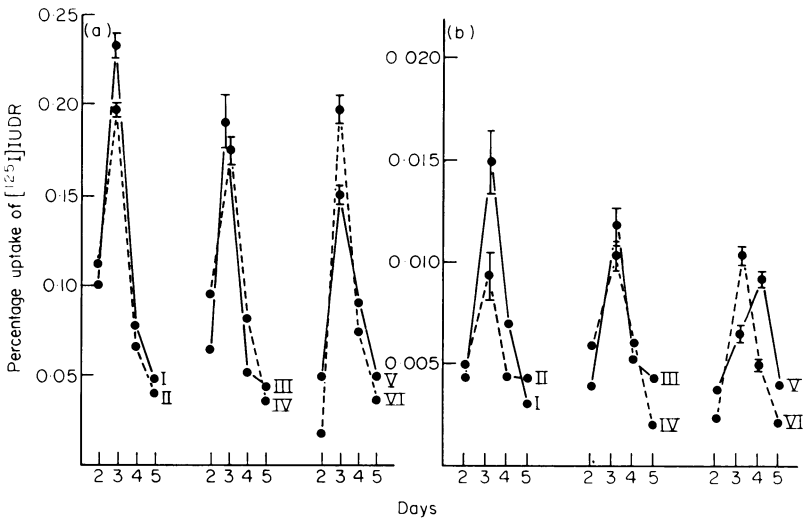

FIG. 1. The DNA synthetic responses of three doses of parental thymocytes (9×10^7 = I and II, 6×10^7 = III and IV, $4{\cdot}5 \times 10^7$ = V and VI in (a) the spleens and (b) lymph nodes of lethally irradiated recipient F_1 mice in (——) the absence or (– – –) presence of a constant number of F_1 thymocytes ($2{\cdot}5 \times 10^7$). Each point is the mean response ± SE of three test mice.

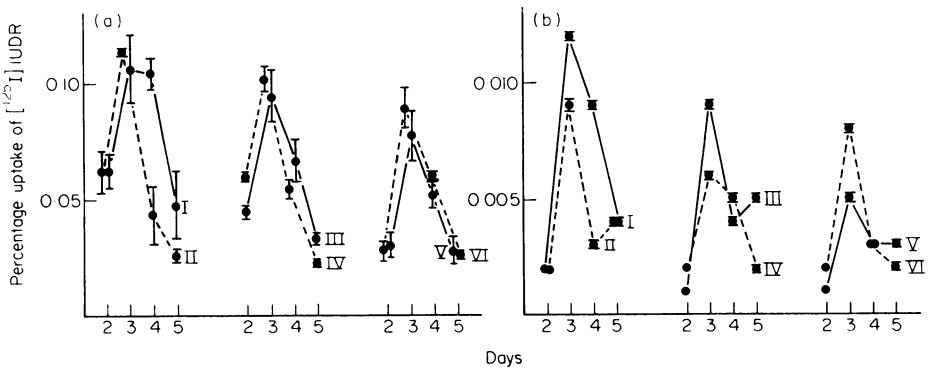

FIG. 2. The DNA synthetic responses of three doses of parental thymocytes (3×10^7 = I and II, 2×10^7 = III and IV, $1{\cdot}3 \times 10^7$ = V and VI) in (a) the spleens and (b) lymph nodes of lethally irradiated recipient F_1 mice in (——) the absence or (– – –) presence of a constant number of F_1 thymocytes ($7{\cdot}5 \times 10^6$). Each point is the mean response ± SE of three test mice.

In both experiments (Figs 1 and 2) the F_1 cells significantly suppressed the response of the highest dose of parental cells and boosted the response of the lowest. Their effect at the middle dose was either nil or intermediate. The changes were most marked in the spleens in the first experiment and in the lymph nodes in the second. As a consequence of these

bidirectional effects of the F_1 cells, the DNA synthetic response of all three doses of parental cells was the same in the presence of the F_1 cells, although the higher doses were always more active than the lower in the absence of the F_1 cells. Thus, the effect of the F_1 cells was to drive all the responses towards the middle.

These experiments pose an interpretative problem. Although we know that F_1 thymocytes do not synthesize significant amounts of DNA when inoculated by themselves into lethally irradiated syngeneic mice, it is possible that the presence of responding parental cells may cause them to do so. Thus, we do not know whether the increased DNA synthetic response seen at the low cell doses was due to the F_1 cells turning on the parental cells or vice versa. To try to determine which was happening we tested the ability of F_1 cells rendered incapable of DNA synthesis, by 900 R of irradiation, to affect the response of untreated parental cells.

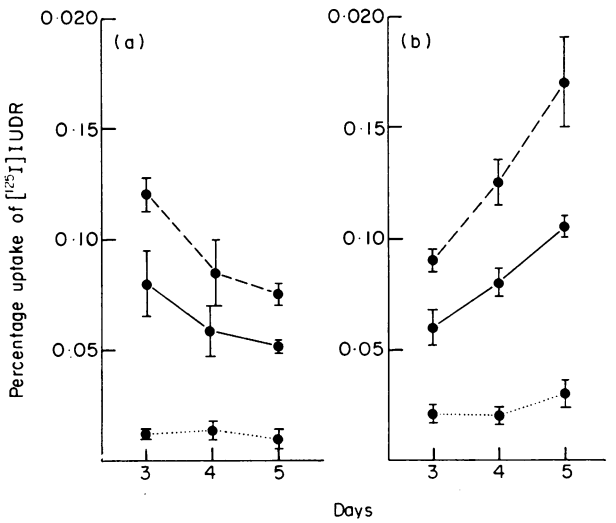

Fig. 3. The DNA synthetic response of $2{\cdot}5 \times 10^7$ parental thymocytes in (a) the spleens and (b) lymph nodes of lethally irradiated F_1 mice (——) in the absence of added cells or (– – –) in the presence of 1×10^7 added normal F_1 thymocytes or (· · · · ·) 1×10^7 irradiated F_1 thymocytes. Each point is the mean response ± SE of three test mice.

EFFECT OF IRRADIATION OF F_1 THYMOCYTES ON THEIR ABILITY TO REGULATE THE RESPONSE OF PARENTAL CELLS

In this experiment we inoculated a single dose ($2{\cdot}5 \times 10^7$) of C_3H thymocytes into four groups of nine lethally irradiated CDF_1 mice. One group got these cells alone, one group got in addition 1×10^7 normal CDF_1 thymocytes, a third the same number of CDF_1 thymocytes which had been irradiated with 900 R, and a fourth 2×10^6 normal F_1 thymocytes. The response of the last group of mice was indistinguishable from the second so only the responses of groups 1, 2 and 3 are presented in Fig. 3. It can be seen that the addition of untreated F_1 cells led to a significant increase in the response ($P<0{\cdot}01$) while the irradiated cells caused a marked depression ($P<0{\cdot}001$).

This experiment suggested to us that there are radiation-resistant regulatory F_1

thymocytes and that radiation-sensitive F_1 cells either (a) proliferate themselves and thus obscure the effect the radiation-resistant cells have or (b) contribute themselves in the regulatory interactions to change the net effect or direction of the regulation, or both. Before either of these hypotheses could be tested it was important to rule out the possibility that the suppression produced by the irradiated F_1 thymocytes was caused by toxic substances released by the dying irradiated cells. To check this possibility we compared the effects of irradiated parental thymocytes with those of irradiated F_1 thymocytes on the DNA synthetic response of normal parental cells in lethally irradiated F_1 mice.

EFFECT OF ORIGIN OF ADDED IRRADIATED THYMOCYTES ON THE DNA SYNTHETIC RESPONSE OF PARENTAL THYMOCYTES

We inoculated, as in the previous experiment, nine lethally irradiated CDF_1 mice with $2{\cdot}5 \times 10^7$ C_3H thymocytes, nine with these cells plus 1×10^7 lethally irradiated C_3H cells

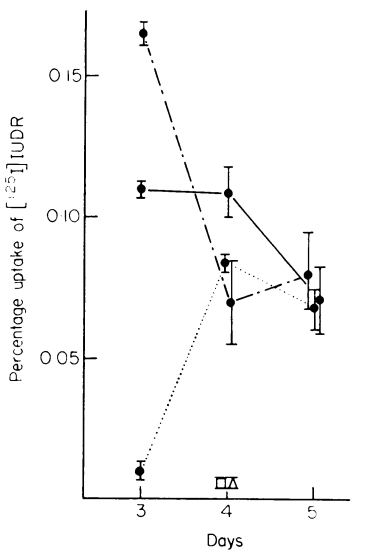

FIG. 4. The DNA synthetic response of $2{\cdot}5 \times 10^7$ parental thymocytes in the spleens of lethally irradiated F_1 mice (——) in the absence of added cells or (· · · · ·) in the presence of 1×10^7 added irradiated F_1 ($Tp+RTF_1$) or (– · –) 1×10^7 parental ($Tp+RTp$) thymocytes. (□) RTF_1. (△) RTp. Each point is the mean response ± SE of three test mice.

and nine with 1×10^7 lethally irradiated F_1 thymocytes added to the inoculum of normal parental thymocytes. The results (Fig. 4) show that the addition of irradiated F_1 thymocytes produced a marked, but temporary, suppression of the parental cell response ($P<0{\cdot}001$), while the addition of irradiated parental cells produced a significant boost ($P<0{\cdot}01$). These results indicate that the suppressive influence of irradiated thymocytes is affected by influences over and above non-specific release of substances by dying cells. They further suggest that irradiated parental cells can release factors which activate other cells. To substantiate the latter point we tested the ability of irradiated parental cells to cause F_1 thymocytes to synthesize DNA in lethally irradiated F_1 mice.

EFFECT OF IRRADIATED PARENTAL THYMOCYTES ON THE DNA SYNTHESIS OF F_1 THYMOCYTES IN IRRADIATED F_1 MICE

We inoculated twelve lethally irradiated CDF_1 mice with 2×10^7 syngeneic thymocytes and twelve with these cells plus an equal number of irradiated C_3H thymocytes. We also gave four mice the irradiated C_3H cells alone. The F_1 thymocytes synthesized very little DNA on their own (Fig. 5) but were stimulated quite a bit by the presence of the irradiated parental cells ($P<0{\cdot}01$). Thus, irradiated parental cells can activate cells which are not by themselves responding to the host antigens. This observation substantiates the findings presented in Fig. 4 and has a significant bearing on the observations presented in Figs 1–3. Effects on the DNA synthesis of parental cells, produced by F_1 cells, can be obscured by proliferation of the F_1 cells due to effects upon them of the parental cells, an interesting circular situation.

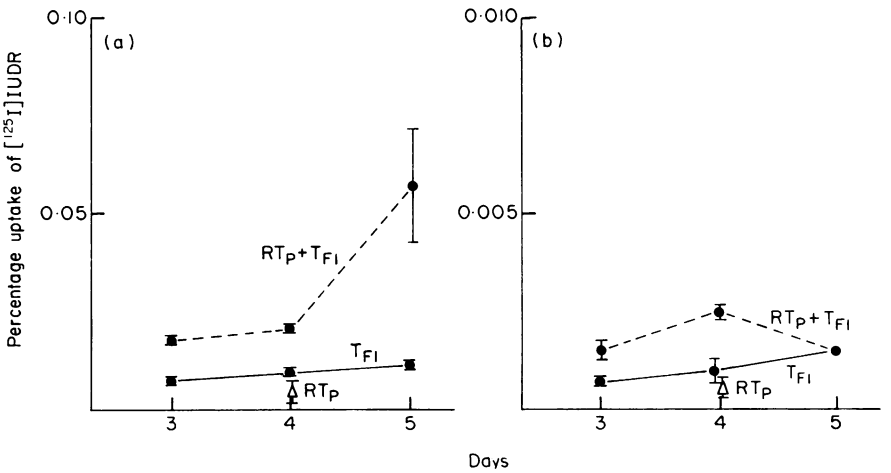

FIG. 5. The DNA synthetic response of 2×10^7 F_1 thymocytes in (a) the spleens and (b) lymph nodes of lethally irradiated syngeneic F_1 mice in (——) the absence or (– – –) presence of 2×10^7 900 R-irradiated parental thymocytes. (△) The response of the irradiated parental cells alone on day 4 is also shown. Each point is the mean response ± SE of four test mice.

EFFECT OF THE ADDITION OF IRRADIATED PARENTAL CELLS TO INOCULA OF NORMAL PARENTAL CELLS, IN THE PRESENCE OR ABSENCE OF F_1 THYMOCYTES

The experiments reported above have shown that parental thymocytes given 900 R *in vitro* do not synthesize DNA in lethally irradiated F_1 mice, but can boost the response of unirradiated thymocytes (either of parental (Fig. 4) or of F_1 (Fig. 5) origin). In addition they have shown that increased responsiveness of an inoculum of parental cells may lead to increased suppression of their response by a given dose of F_1 thymocytes (Figs 1 and 2). We therefore reasoned that, if the irradiated parental thymocytes could increase the response of the normal parental cells, and if the increased activity of these cells was responsible for the suppressor activity, mediated by F_1 cells, then the addition of irradiated parental cells to normal parental cells should have a suppressive effect in the presence of F_1 thymocytes. We tested this postulate in the following manner. We inoculated twenty-

four lethally irradiated CDF_1 mice with 2×10^7 normal C_3H thymocytes and another twenty-four with these cells plus 2×10^7 CDF_1 thymocytes. Half of each group was also given 2×10^7 900 R-irradiated C_3H thymocytes. In these experiments the DNA synthetic response of the normal parental thymocytes was not affected by the F_1 thymocytes in the F_1 recipients which had got no other cells (Fig. 6), but was suppressed ($P<0{\cdot}01$) by the F_1 cells in the recipients which were given the additional 900 R-irradiated parental cells. Significantly, in the latter group of mice the response of the normal parental cells was increased in the absence of F_1 thymocytes ($P<0{\cdot}02$) while it was decreased on day 3 ($P<0{\cdot}05$) in the presence of the F_1 thymocytes. These results confirm the general notion that the activity of a responding cell population is an important factor in determining the direction of the regulation effected by F_1 thymocytes in the F_1 mice, and the specific notion that increased activity of the responding cells results in increased suppression.

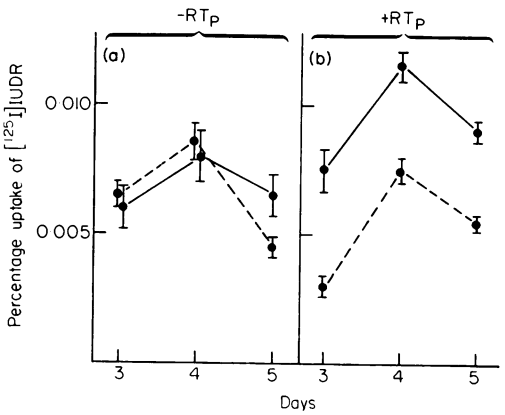

FIG. 6. (a) The effect of 2×10^7 900 R-irradiated parental thymocytes (+RTp) on the DNA synthetic response of 2×10^7 normal parental thymocytes in the lymph nodes of lethally irradiated F_1 mice in (——) the absence or in (– – –) the presence of 2×10^7 added normal F_1 thymocytes. Each point is the mean response ± SE of four test mice. (b) In the absence of RTp.

BOOSTING OF THE RESPONSE OF NORMAL PARENTAL THYMOCYTES WITH IRRADIATED F_1 THYMOCYTES

The experiments reported so far indicate that under the proper conditions 900 R-irradiated F_1 cells can suppress the DNA synthetic response of normal parental cells and that 900 R-irradiated parental cells can augment it. It remains to be seen if conditions can be found where irradiated F_1 cells augment the response and irradiated parental cells suppress it.

The results presented in Fig. 7 indicate that F_1 thymocytes irradiated with 900 R can boost the response of normal parental thymocytes. In that experiment we inoculated four groups of nine F_1 mice with $2{\cdot}5 \times 10^7$ parental thymocytes. One group got those cells alone, one got in addition 1×10^7 normal parental thymocytes, the third 1×10^7 parental thymocytes irradiated with 900 R and the fourth 1×10^7 F_1 thymocytes irradiated with 900 R. All the groups with added cells made significantly increased responses ($P<0{\cdot}001$). Particularly striking in this experiment was the augmentation produced by 1×10^7 normal parental thymocytes in the lymph nodes. The 40 per cent increase in cell dose

produced up to a 500 per cent increase in DNA synthetic activity. This 'multiple order' type reaction has been seen occasionally before (Gershon and Liebhaber, 1972) and may help to explain why the irradiated F_1 thymocytes boosted the response in this experiment while they had suppressed it previously. The parental cells alone were responding poorly for the numbers of cells injected, perhaps due to self-regulatory mechanisms, and thus, the balance was easily tipped upward by the added cells. It is unfortunate that we did not add F_1 cells to the highly responding group as it would have been instructive to see if they would have been suppressive under those conditions, as they were in the boosted response reported in Fig. 6. These results demonstrate that conditions may exist where irradiated F_1 cells can boost the response of parental cells in F_1 mice.

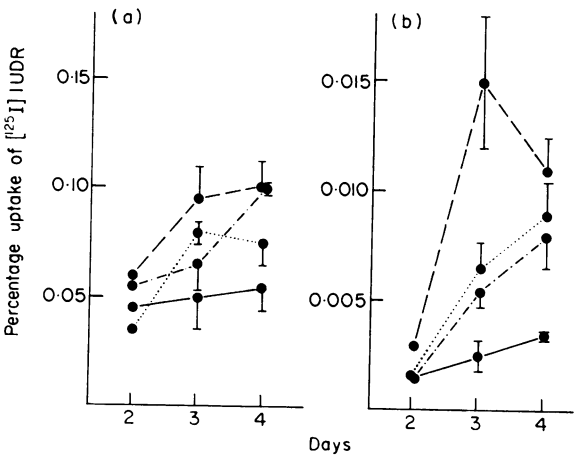

FIG. 7. The DNA synthetic response of $2{\cdot}5 \times 10^7$ parental thymocytes in the spleens and lymph nodes of lethally irradiated F_1 mice (——) in the absence of added cells or (– – –) in the presence of 1×10^7 added normal parental thymocytes, or (– · –) 1×10^7 900 R-irradiated parental thymocytes or (· · · ·) 1×10^7 900 R-irradiated F_1 thymocytes. Each point is the mean ± SE of three test mice.

BIDIRECTIONAL REGULATORY EFFECTS OF 900 R-IRRADIATED PARENTAL THYMOCYTES ON THE RESPONSE OF NORMAL PARENTAL THYMOCYTES IN F_1 MICE

We have shown that varying the activity of an inoculum of parental thymocytes into a lethally irradiated F_1 host causes the addition of F_1 thymocytes to that inoculum to have an opposite effect: when the activity of the parental cells is high, the F_1 cells suppress the response and when it is low they boost it. We have varied the activity of the parental cells in two ways: (1) varying the cell dose; and (2) boosting a given dose of cells by the addition of 900 R-irradiated parental cells which are unable by themselves to synthesize DNA.

We have tried a third method of varying the parental cell activity. After harvesting the thymocytes we gave an aliquot 20 R of irradiation and another 100 R. A third aliquot was unirradiated. We added to half of each aliquot an equal number of parental cells which had received 900 R. We injected groups of twelve lethally irradiated F_1 mice with one of the above cell suspensions. Thus, recipient mice got 4×10^7 parental thymocytes given either 0 R, 20 R or 100 R with or without an additional 4×10^7 cells which were irradiated with 900 R. The results (Fig. 8) show that 100 R of irradiation produced a

significant fall in the DNA synthetic activity of the parental thymocytes responding in the absence of added 900 R cells ($P<0{\cdot}001$). The 100 R also changed the regulation effected by the added 900 R cells. These cells suppressed the activity of the untreated cells ($P<0{\cdot}01$) and boosted the activity of the cells treated with 100 R ($P<0{\cdot}01$). Their effect on the 20 R-irradiated cells was intermediate. As a result, the progressive fall in activity produced by the increasing radiation dose given to the parental cells was either abolished (spleens) or actually reversed (lymph nodes) by the addition of lethally irradiated cells. These results are strikingly similar to those produced by varying the dose of normal parental cells in the presence or absence of added F_1 thymocytes (Figs 1 and 2) and give further evidence that the regulatory effects of non-responding thymocytes are determined to some degree by the activity of the responding cells. They also show that irradiated parental cells, in a fashion similar to irradiated F_1 cells, can suppress as well as augment the response of normal parental thymocytes.

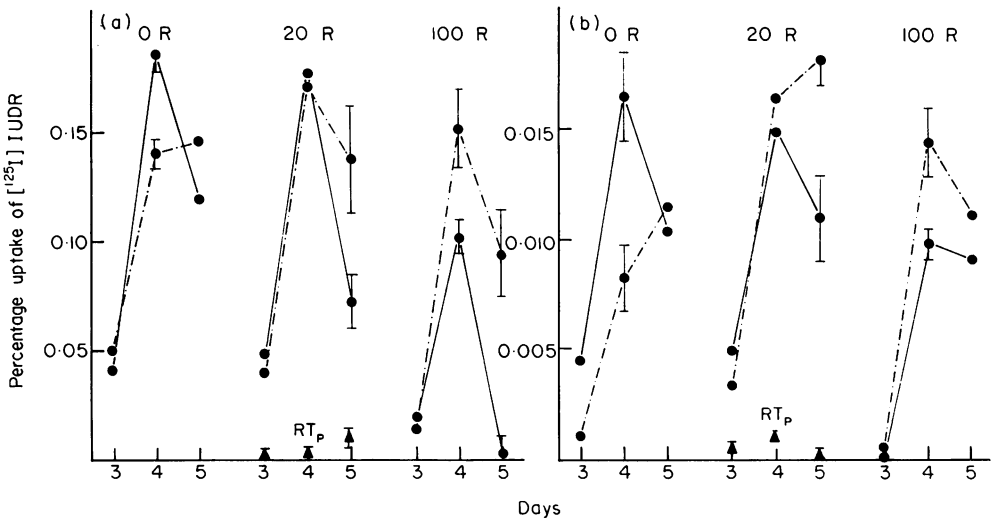

FIG. 8. The DNA synthetic response of 4×10^7 parental thymocytes in (a) the spleens and (b) lymph nodes of lethally irradiated F_1 mice. The thymocytes were treated with 20 R or 100 R or were untreated (0R) prior to inoculation. Their response in (——) the absence or (– · –) the presence of 4×10^7 900 R-irradiated parental thymocytes is presented. In addition the responses of (▲) the 900 R-irradiated cells is also presented. Each point is the mean response + SE of four test mice.

DISCUSSION

The results we have presented indicate that thymocytes which are not responding to antigenic stimulation can regulate the response of thymocytes which are. Thus, the addition of F_1 thymocytes to inocula of parental thymocytes in lethally irradiated F_1 mice can significantly alter the parental cell response. In this situation the F_1 cells have no antigen to respond to and indeed do not synthesize measurable amounts of DNA when inoculated by themselves. The most likely interpretation of these observations is that the F_1 thymocytes act in response to signals given to them by the parental thymocytes, and in return signal the parental cells in a fashion that affects the parental cell response.

There is considerable evidence in the literature that the response of T cells to histocompatibility antigens results in the production of signals to bystander cells. A number of workers have demonstrated that the introduction of parental cells into F_1 mice results in proliferation of the hosts cells (Biggs and Payne, 1959; Davies and Doak, 1960; Fox, 1962; Zeiss and Fox, 1973; Nowell and Defendi, 1964; Owen *et al.*, 1965; Nisbet and Simonsen, 1967; Nakic *et al.*, 1967; Elkins, 1970). The 'allogeneic effect' is another example where the response of T cells to histocompatibility antigens activates bystander cells (Katz, Paul, Goidl and Benacerraf, 1971). In this case the bystander cells are otherwise unresponsive B cells which can be induced to make antibody in the presence of antigen and signals from T cells producing a GVHR. Such T-cell influences can prevent the formation of (Gershon and Kondo, 1972) and even break an established state of immunological tolerance (McCullagh, 1970). Thus, there is adequate precedent for the stimulation of F_1 cells by parental thymocytes. Indeed, we directly demonstrated such an occurrence in our experiments (Fig. 5). It should therefore not be surprising that the secondarily responding cells should themselves transmit signals to the cells which stimulated them and in so doing, influence their response.

We showed that the influence the F_1 cells had on the responding parental thymocytes was determined, at least in part, by the level of responsiveness of the parental cells themselves; a constant dose of F_1 cells suppressed the response of a high dose of thymocytes and boosted the response of a lower dose. The F_1 cells had an intermediate effect on an intermediate dose of parental thymocytes.

Although the F_1 thymocytes in these experiments had no antigen to respond to, they acted as a source of antigen for the parental cells and may have affected their response by virtue of this attribute. Many inferential arguments can be offered that militate against this possibility as the main cause of the observed results, but it cannot unequivocally be ruled out. However, we were also able to show that parental thymocytes, rendered incapable of DNA synthesis by 900 R of irradiation, could produce the same effects on DNA synthesizing parental cells as could F_1 cells. In this case the regulating cell population did not act as a source of antigen. In contradistinction to the F_1 cells however, the 900 R-irradiated cells did have a source of antigen to react to themselves (the F_1 host). Nonetheless, the regulatory effects they produced were strikingly similar to those produced by the F_1 cells. Thus, a constant dose of 900 R-irradiated parental thymocytes suppressed the response of a high dose of thymocytes and boosted the response of the same thymocytes which had been reduced in number and activity by 100 R of irradiation. Treatment of the responding thymocytes with 20 R also altered the effect the 900 R cells had, but not as dramatically.

Assuming the regulation effected by the F_1 thymocytes and the 900 R-irradiated parental thymocytes has the same basic mechanism it would seem that the T-cell response to antigen is composed of a sequence of events. An antigen-reactive cell transmits a signal to another cell, which may or may not also be antigen-reactive. As a consequence of that signal the second cell responds and in so doing transmits a signal back to the antigen-reactive cell which affects its response. When only a few cells are responding the signal from the second cell is a positive one. That is, it augments the response. This probably accounts for the dose-dependent latent period which precedes the DNA synthetic response of T cells to antigen (Gershon and Liebhaber, 1972). This latent period is considerably shortened if the number of responding cells in increased. This suggests an augmenting interaction between the inoculated cells early in the response. When the response

builds up the signals received by the non-responding cell are translated in such a way as to cause them to signal the responding cells to cease their response, resulting in a shut-off effect. This explains why increasing the number of responding cells results in an earlier shut-off of the response (Gershon and Hencin, 1971; Gershon and Liebhaber, 1972); long before the full potential of the response has been realized. It is not necessary to postulate that the different effects the cell interactions produce are caused by different substances. Different amounts of the same substance could explain the results equally well. That is, weakly responding cells may be making only small amounts of a stimulatory factor and are therefore helped when other cells are making it too. When both cells make a lot of the factor however, it may then act in an inhibitory fashion. Although we have not as yet examined the T-cell response in lethally irradiated mice in the period after 5 days post-inoculation, owing to technical difficulties (Gershon and Hencin, 1971), we would suspect that when the signals of the responding cells abated, due to the cessation of response, the second cell might begin sending positive signals again, as it did when the response was low and the cycle recommenced.

It has been clearly demonstrated that several aspects of the immune response are composed of such cycles of response and non-response (Möller, Britton and Möller, 1968; Simpson and Beverly, 1972). Antibody feedback has been suggested to be the cause. Our work indicates that feedback products of cells other than B cells may also be operative. It is possible that some of the feedback effects of antibody are mediated through T cell–T cell regulatory interactions, similar to those we describe. Thus, antigen–antibody complexes in antigen excess might cause the regulatory T cell to augment the response while in antibody excess the signal would be a negative one.

The role of macrophages in the mediation of the interaction effects we have described is unknown. Macrophage reactions are known to be under T-cell control (Mackaness, 1969) and T cells are also known to be affected by macrophage products (Bach, Alter, Solliday, Zoschke and Janis, 1970; Gery, Gershon and Waksman, 1972; Gery and Waksman, 1972). Macrophages (reviewed by Chang and Hirsch, 1972) as well as soluble products isolated from them (Hoffman and Dutton, 1971) have been shown to be capable of both augmentation and suppression of *in vitro* immune responses. Therefore, the radiation-resistant macrophages in the irradiated T-cell recipients may have indirectly mediated some or all the T-cell interactions we described. Sjöberg has recently put forth evidence for such indirect T-cell mediated reactions (Sjöberg, 1972). We would think it likely that all cells under T-cell control, which include B cells (Miller and Mitchell, 1969; Davies, 1969; Claman and Chaperon, 1969; Gershon and Kondo, 1970), macrophages (Mackaness, 1969), eosinophils (Basten and Beeson, 1970) and basophils (Dvorak, Dvorak, Simpson, Richerson, Leskowitz and Karnovsky, 1970) would have a mechanism for signalling the T cell in a regulatory fashion.

At least two T-cell subpopulations have recently been defined (Asofsky, Cantor and Tigelaar, 1971; Cantor and Asofsky, 1970; Cantor and Asofsky, 1972; Tigelaar and Asofsky, 1972; Tigelaar and Asofsky, 1973) and christened T_1 and T_2 (Raff and Cantor, 1971). We cannot tell from the present experiments how interactions between these two cell populations affected the regulation we observed, although previous experiments with cells fractionated by sensitivity to cortisone (Cohen and Gershon, submitted for publication) and splenic localization (Gershon, Lance and Kondo, 1974) suggest that such interactions play a significant role.

Our demonstration that 900 R-irradiated parental thymocytes can either potentiate the

response of normal parental thymocytes in F_1 mice or actually cause the F_1 thymocytes to react stands in contrast to several failures to demonstrate F_1-cell activation by parental thymocytes in mixed lymphocyte reactions (MLR) (Jones, 1972; Wilson, Silvers and Nowell, 1967). However, other workers have been able to demonstrate F_1-cell responses (Adler, Takiguchi, Marsh and Smith, 1970) and the release of mitogenic factors (Gery, Gershon and Waksman, 1972; Kasakura and Lownstein, 1965) *in vitro*. Since parental cell activation of F_1 cells *in vivo* seems to be a general finding, it is possible that some *in vitro* conditions are not optimal for the demonstration of these interactions. If the F_1 cell activation is an indirect one, mediated by macrophage released factors (see above), a lack of these cells *in vitro* could be a factor.

The ability of heavily irradiated T cells to participate in immune responses has been previously described by a number of workers (Katz, Paul, Goidl and Benacerraf, 1970; Kettman and Dutton, 1971; Munro and Hunter, 1969; Hirst and Dutton, 1970; Playfair and Marshall-Clarke, 1973) although others have found T cells to be quite radiosensitive (Claman and Chaperon, 1969; Ito and Cudkowicz, 1971; Anderson, Sprent and Miller, 1972). The reasons for these discrepancies are not clear. Factors such as the effect of pre-immunization, source of cells, *in vitro versus in vivo* activity and mode of assay have not been systematically tested. This is the first report to suggest that heavily irradiated, thymic T cells may participate in an immune response. It also is the first reported study in which the effects of the irradiated thymocytes were tested in the presence of unirradiated normal T cells. Previous studies measured the effect of the irradiated cells on isolated B cells (Claman and Chaperon, 1969; Ito and Cudkowicz, 1971; Anderson, Sprent and Miller, 1972). Perhaps the need for amplifying T cell–T cell interactions, such as we have reported, was in part responsible for the negative results.

Our demonstration that F_1 thymocytes regulate the response of parental thymocytes in F_1 mice offers a possible explanation for the premium effect described by Celada (Celada, 1966) and for other instances where irradiation of recipients of immunologically competent cells allows the transferred cells to make a better response (Simonsen, 1962; Elkins, 1971). Since the irradiation of the recipients does not appear to increase the localization of the inoculated cells (Gershon, Cohen, Hencin and Liebhaber, 1972), elimination of regulating F_1 cells by the irradiation is a possible alternative explanation. If F_1 T cells play an important role in this regulation, their relative absence in new-born animals would also explain why adoptively transferred cells perform much better in them than in adults (Celada, 1966; Simonsen, 1962; Elkins, 1971).

At first glance it seems puzzling that 900 R-irradiated F_1 thymocytes should produce such striking regulatory effects, when added to F_1 mice irradiated with 900 R. These mice should contain large numbers of cells similar to the ones that were added. Possible explanations for this apparent paradox include: (1) the residual irradiated F_1 cells of the recipients do indeed act in a regulatory fashion but that the added F_1 cells act in addition to or in concert with them to change their effect; (2) F_1 thymocytes have a different regulatory capacity from peripheral lymphocytes and these cells are not present in the tissues of F_1 mice unless put there by the experimenter. We are presently testing these hypotheses and preliminary evidence indicates that both may be acceptable. In addition we are testing the regulatory capacity of cells other than thymocytes.

In conclusion, the results we have presented indicate that T cells can respond to signals from other T cells and that the secondarily responding T cells can, in return, affect the response of the cells from which they received the signals.

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