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# Strain Differences in the Immune Response of Mice

## II. RESPONSES BY NEONATAL CELLS IN IRRADIATED ADULT HOSTS

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**Summary.** Lethally irradiated NZB and C57BL mice were injected with syngeneic thymus and marrow, or thymus and liver cells, and immunized with sheep red blood cells (SRBC). As in intact neonatal mice, NZB plaque-forming cell (PFC) responses were significantly higher than C57BL.

Irradiated (NZB × C57BL)$F_1$ and (NZB × BALB/c)$F_1$ hybrid mice were given mixtures of parental and syngeneic cells, and it was shown that the high NZB response to SRBC was characteristic of the newborn liver rather than of the thymus. NZB liver gave rise to more PFC against SRBC than BALB/c liver, but not more against chicken RBC. It is argued that liver cells contain genetic information regarding antibody specificity.

C57BL cells grew poorly in $F_1$ hosts, but the low SRBC response appeared to be characteristic of both liver and thymus.

The nomenclature of the participating cells, and their role in the development of immunological responsiveness, are discussed.

## INTRODUCTION

In a previous paper (Playfair, 1968) it was reported that inbred strains of mice differ markedly in the rate at which their haemolytic antibody response to sheep red blood cells (SRBC) develops in the immediate post-natal period. NZB mice were earlier, and C57BL later, than four other strains. The difference appeared to be determined by several genes, and to depend on the presence of the thymus. While the early NZB response seemed to be specific for SRBC, the belated C57BL response also extended to other erythrocyte antigens.

The present study was an attempt to discover when and where these strain differences arise, by testing individually and in combination the foetal and neonatal organs thought to be involved in the establishment of the humoral immune system—liver and thymus. Advantage was taken of the ability of lethally irradiated adult mice to support the growth and differentiation of antibody-forming cells and their precursors. Most of the experiments exploited the discovery made by Claman, Chaperon and Triplett (1966) that cells from bone-marrow and thymus, injected with SRBC into irradiated mice, interact to produce more antibody together than separately. It will be shown that foetal and neonatal liver can replace bone-marrow in this interaction, and that the strain differences previously described in intact neonatal animals are already embodied before birth in the liver, and in some cases, also in the thymus. Comparisons will be drawn between the responses to SRBC and chicken RBC, and between syngeneic and semi-syngeneic cell transfers.

## MATERIALS AND METHODS

*Mice*

The strains used were NZB, BALB/c, C57BL, (NZB × C57BL)$F_1$, and (NZB × BALB/c)$F_1$. Details of origin, breeding, and care were given previously (Playfair, 1968). When 19-day-old foetal mice were required, females were mated for one night only.

*Irradiation*

X-rays were generated by a Marconi 250 kVP CP Therapy Control Unit, operated at 230 kVP with 1 mm Cu and 1 mm Al added filtration. Mice were irradiated at 3–4 months of age, inbred strains receiving 850 rad and $F_1$ hybrids 900 rad, at a dose rate of 23–25 rad/min.

*Cell suspensions*

Bone-marrow was blown from cut femurs with Eagle's medium via a 21 gauge needle. Thymuses and livers were cut in two and teased in Eagle's medium with broad flat forceps; the capsules and stroma were removed and the cells gently drawn through 25 gauge needles. Thymus cells were washed once in cold Eagle's medium, liver cells twice (10 minutes at 150 ***g***). Nucleated cells were counted and the desired number injected via the tail vein in 0·3 ml, about 4 hours after irradiation. At the same time, and again 3 days later, $4 \times 10^8$ SRBC or chicken RBC were injected intraperitoneally.

*Antibody detection*

Eight days after irradiation, spleens were removed and teased in Eagle's medium, and antibody plaque-forming cells (PFC) measured by the standard method of Jerne, Nordin and Henry (1963).

*Iron uptake*

$^{59}Fe$, as high specific-activity ferric citrate (Radiochemical Centre, Amersham) was injected intravenously 4 hours before the mice were killed, and the counts in the spleen expressed as a percentage of a standard representing the injected dose.

## RESULTS

### SYNGENEIC CELL TRANSFER

In the first group of experiments, NZB and C57BL mice were irradiated, injected with syngeneic cells, immunized with SRBC and their spleen PFC counted. In each experiment, batches of mice were given: (a) no cells, (b) $15 \times 10^6$ newborn (0–7-day-old) thymus cells, (c) $15 \times 10^6$ newborn (0–5-day-old) liver cells, or (d) liver and thymus cells together. The distributions of the resulting PFC counts are shown in Fig. 1. In both strains thymus cells alone produced little or no increase above the no-cell 'background'. In the NZB, liver cells caused a small but significant increase, while liver plus thymus gave very high PFC counts. Even assuming that the PFC due to 'background', and to thymus and liver cells alone, were also present in the liver-plus-thymus group, and subtracting them from the total, there were still some 11,000 'new' PFC/spleen. In the C57BL, however, the corresponding figure would be only about 800 'new' PFC. In other

words, the large difference previously noted between the PFC responses in the neonatal NZB and C57BL spleens was reproduced when newborn liver and thymus cells were allowed to interact in the irradiated adult spleen.

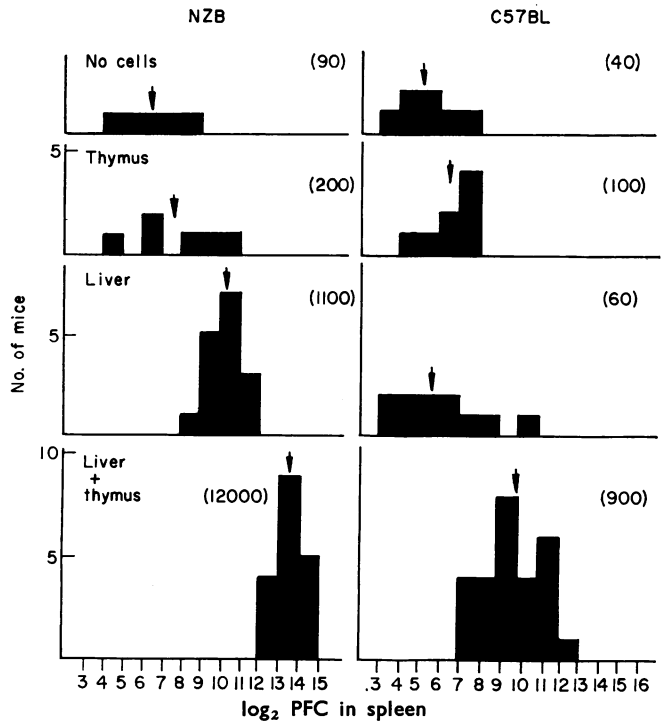


FIG. 1. Distribution of 8-day spleen PFC counts in NZB and C57BL mice irradiated, injected with $15 \times 10^6$ newborn syngeneic thymus and/or liver cells, and immunized with SRBC. The arrows and the numbers in parentheses indicate the logarithmic means.

To check that a general failure of C57BL cells to repopulate the irradiated adult spleen was not to blame for their low PFC counts, some of the liver-injected mice were given, instead of SRBC, an intravenous injection of $^{59}Fe$, and killed 4 hours later. Whether 3, 5 or 8 days had elapsed since irradiation, the amount of iron incorporated into the spleens of NZB and C57BL was similar, showing that at least the erythropoietic cells were proliferating satisfactorily in both strains

*Effect of donor age*

The liver-plus-thymus results, which are pooled in Fig. 1, were analysed with respect to the age of the donor. When newborn liver was used, variations in the age of the *thymus* donor made little difference, $15 \times 10^6$ thymus cells from 0- to 7-day-old mice being equally effective, although, since the size of the thymus increases rapidly during this period, the total activity per gland (which was not tested) would no doubt show an increase. But when the *liver* was taken from older donors there was a progressive fall in the 'new' PFC resulting from the addition of thymus cells. In one experiment, 19-day-old foetal liver was much less effective than newborn (Fig. 2). Since the average nucleated cell yield per liver also

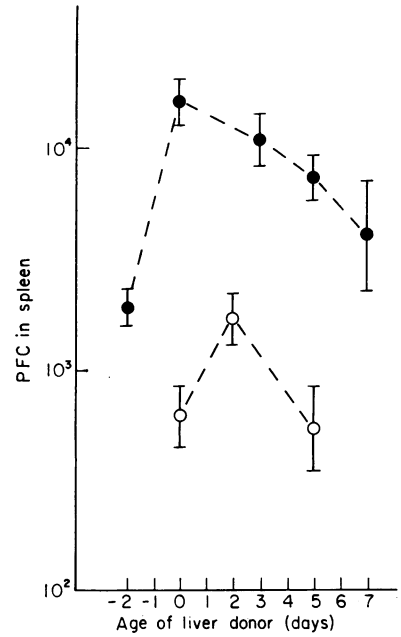


FIG. 2. Eight-day spleen PFC counts in NZB (●) and C57BL (○) mice irradiated, injected with syngeneic newborn thymus cells, and liver cells from donors of various ages, and immunized with SRBC. The values shown are the logarithmic means ± 1 SE.

declined, from about $25 \times 10^6$ at birth to $15 \times 10^6$ at 7 days, the total contribution of the liver probably falls more steeply than Fig. 2 suggests. After 7 days it is doubtful if the addition of thymus cells produces any significant amount of 'new' PFC. In the C57BL, 2-day liver was somewhat better than newborn, but thereafter the same decline was seen.

BALB/c mice were not used for these syngeneic experiments on account of their unusually low radio-resistance.

## CELL TRANSFER TO $F_1$ HYBRIDS

The above experiments showed that the neonatal difference in SRBC responsiveness was a property of neonatal *cells* rather than their neonatal *environment*, but did not establish whether the difference resided in the liver or the thymus or both. For this, it was necessary to test liver and thymus cells separately. The method chosen was to grow and challenge them in $F_1$ hybrid mice in which one cell type—liver (marrow) or thymus—was also of $F_1$ origin, and therefore syngeneic with the host, while the other came from one of the parental strains. In this system it was possible to study BALB/c cells as well as NZB and C57BL, since $F_1$ hybrids all withstand irradiation well. The results in the two strain combinations will be described separately.

### *NZB and C57BL*

Fig. 3 shows the PFC distributions in the irradiated (NZB × C57BL) $F_1$ mice. Three points are noteworthy:

(1) In the fully syngeneic ($F_1$) combination, liver and marrow gave identical average

counts. This justified the use, when thymus cells were being tested, of adult marrow instead of newborn liver, which is not so readily available.

(2) When either NZB liver *or* NZB thymus were substituted for the corresponding $F_1$ tissue, the average PFC counts increased about three-fold.

(3) When C57BL thymus was substituted, the average PFC decreased two-fold, whereas when C57BL liver was substituted, PFC counts fell to normal 'background' values.

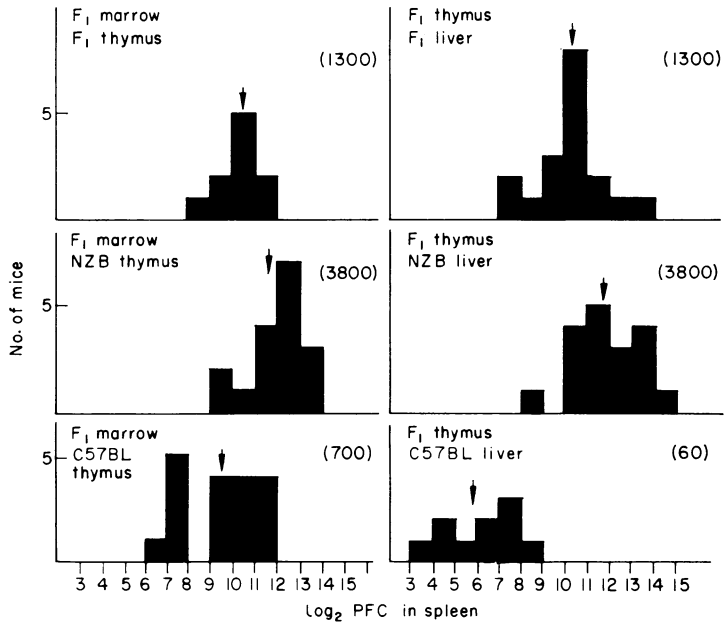


FIG. 3. Distribution of 8-day spleen PFC in (NZB × C57BL)$F_1$ mice irradiated, injected with newborn thymus and liver or adult bone-marrow from syngeneic or parental donors, and immunized with SRBC. Details as in Fig. 1.

At face value, these results suggest that the major deficiency was in the C57BL liver. However, when the 4-hour spleen iron uptake was measured in the various groups, it was found that C57BL liver gave extremely low counts, denoting a poor repopulation by haemopoietic cells. This observation confirms those of other investigators (Popp and Cudkowicz, 1965) that C57BL haemopoietic cells grow badly in $F_1$ hybrid hosts, and will be returned to in the discussion. It was not possible to be sure whether all the PFC reduction with the C57BL liver was due to this effect, or whether the same effect caused the smaller reduction with C57BL thymus.

NZB liver, however, gave good haemopoietic repopulation, though a little less than $F_1$ liver. Therefore, the high NZB results can probably be treated as valid evidence for a superiority of NZB over $F_1$ cells in both liver and thymus, of about the same degree. Since the $F_1$ response lies between the NZB and C57BL—both in the syngeneic cell transfer system and in the intact neonatal animal—one might anticipate a roughly equal superiority of $F_1$ cells over C57BL; in fact the C57BL thymus result might be quite valid, though the C57BL liver result is no doubt falsely low.

In one experiment, both liver and thymus cells were from NZB donors; surprisingly, the average PFC count was only 1400: lower than with mixed NZB and $F_1$ cells.

*NZB and BALB/c*

In (NZB × BALB/c)$F_1$ hybrids, NZB and BALB/c liver gave good and equal haemopoietic repopulation, which increases confidence in the PFC counts. Fig. 4 shows that, as compared with the fully syngeneic combination, the replacement of $F_1$ thymus by NZB or

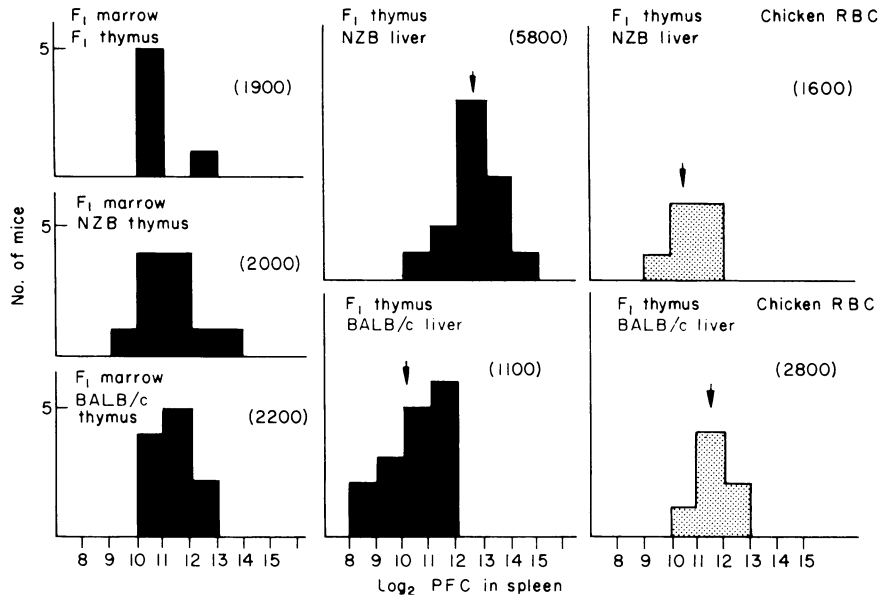


FIG. 4. Distribution of 8-day spleen PFC in (NZB × BALB/c)$F_1$ mice irradiated, injected with newborn thymus and liver or adult bone-marrow from syngeneic or parental donors, and immunized with SRBC or chicken RBC. Details as in Fig. 1.

BALB/c thymus caused no significant change in PFC response. Indeed in one experiment using fewer ($5 \times 10^6$) thymus cells, BALB/c thymus gave *more* PFC than NZB. However, the substitution of NZB liver resulted in about a three-fold rise, and that of BALB/c in a two-fold fall, of PFC counts. Thus it appears that the superiority of NZB over BALB/c in SRBC responsiveness derives chiefly or entirely from the liver cells.

*Chicken RBC*

Also shown in Fig. 4 are some PFC responses to chicken RBC. In this case NZB liver was no better, and possibly a little less good, than BALB/c, in combination with $F_1$ thymus. This closely reflects the situation in intact neonatal mice, where NZB and BALB/c showed no significant difference in their PFC response to chicken RBC (Playfair, 1968). The implication is that the superiority of NZB liver over BALB/c does not embrace all antigens.

*Thymectomized recipients*

In one group of (NZB × C57BL)$F_1$ hybrids, thymectomy preceded the irradiation, and syngeneic liver and thymus cells were injected. The PFC counts were the same as in non-thymectomized mice, and have been pooled with them in Fig. 3.

## DISCUSSION

The main conclusion to be drawn from these experiments is that the strain differences in the neonatal response to red-cell antigens can be resolved into two categories:

(1) The difference between C57BL and the other strains studied, affecting all the antigens so far tested, and apparently associated with differences in both newborn liver and thymus cells.

(2) The difference between NZB and BALB/c, affecting some antigens but not others, and associated with differences in newborn liver but not thymus cells.

Before commenting further, it is necessary to define, as far as possible, the cell types involved.

*Nomenclature*

Where haemopoietic cells are concerned, several systems of nomenclature co-exist uneasily. Some are no more than anatomical (thymocyte, foetal liver cell), while others, though based on light or electron microscopy, carry implications of function (transitional cell, haemocytoblast). Others again are abstract (stem cell, memory cell), or hypothetical (X, Y, Z cells, etc.).

With the introduction of methods for studying antibody formation at the single cell level (Jerne *et al.*, 1963), a new set of descriptive terms was needed, based solely on demonstrable functional properties. 'Antibody-forming cells', of which PFC are a subset, might be defined as cells which, when cultured under suitable conditions, start to release antibody at once; they are most numerous in newly immunized animals, and it is generally assumed that they were already making antibody at the time of removal. A more recent type of assay, at first requiring cell-transfer to irradiated mice (Playfair, Papermaster and Cole, 1965; Kennedy, Siminovitch, Till and McCulloch, 1965) but later carried out entirely *in vitro* (Robinson, Marbrook and Diener, 1967), revealed the existence of cells in non-immunized animals that behave like precursors of antibody-forming cells, responding to antigen by the production, in a matter of days and presumably by cell division, of clones of cells forming the same antibody. These cells have variously been called 'precursor cells' (Playfair *et al.*, 1965), 'antigen sensitive cells' (Kennedy *et al.*, 1965), and 'potentially immunocompetent cells' (Claman *et al.*, 1966). They are principally found in the 'immunologically competent' organs: spleen and lymph node.

A further step was the discovery that cells from the 'immunologically incompetent' bone-marrow and thymus, though separately poor in antibody-forming cell precursors, could generate them when combined in an irradiated host (Claman *et al.*, 1966). The indications are that it is the bone-marrow (or foetal liver) cell, and not the thymus, that furnishes the eventual antibody-forming cell, as judged by chromosome marker (Davies, Leuchars, Wallis, Marchant and Elliott, 1967; Mitchell and Miller, 1968) or immunoglobulin allotype experiments (Tyan, Cole and Herzenberg, 1967). Thus the 'precursor cell' mentioned above is evidently of bone-marrow origin. The role of the thymus cell is less clear. One possibility is that it reacts first with antigen, elaborating something which then activates the bone-marrow cell. If this is so, it is the thymus cell that ought to be called 'antigen sensitive', in which case the terms 'antigen sensitive cell' and 'precursor cell', introduced as synonyms, refer in fact to different types of cell. Alternatively, it might be the marrow cell, under the influence of the thymus, that becomes 'antigen sensitive'. Until the exact sequence of events from the interaction of antigen, thymus cell

and marrow cell to the production of antibody is determined, a less ambiguous terminology seems desirable.

For the purposes of this discussion, the following non-committal terms will be used:

(1) *Liver (or marrow) PFC precursor.* A liver cell that gives rise to PFC only when antigen and thymus cells are also present.

(2) *Thymus PFC inducer.* A thymus cell that enables the liver PFC precursor to produce PFC.

(3) *Spleen PFC precursor.* The cell measured by the spleen-cell transfer systems mentioned above, giving rise to PFC in the presence of antigen. Since in assays both *in vivo* (Playfair *et al.*, 1965) and *in vitro* (Robinson *et al.*, 1967) the correlation between spleen cells and 'clones' of PFC has been linear, it would follow that the spleen PFC precursor is either: (a) a single cell, by definition different from the liver PFC precursor, though probably derived from it, or (b) the combination of a liver PFC precursor and a thymus PFC inducer, one cell type being in sufficient excess for the other to behave as the limiting factor in the dose : response relationship (in the latter case, the name 'spleen PFC precursor' would not actually describe any new type of cell). Roughly, the spleen PFC precursor might be called 'competent' and the liver and thymus cells 'incompetent', though incompetent in different ways.

The present experiments bring out certain features of these cell types:

(1) The *liver PFC precursor* is most numerous at or just after birth, declining in numbers at the time when the spleen PFC response (and, therefore, presumably the spleen PFC precursor population) is increasing. The simplest explanation would be that these cells leave the liver and colonize the spleen. Possibly they may go via the thymus, but they can evidently get to the spleen directly, as in the thymectomized, irradiated $F_1$ hybrids already mentioned. Another type of liver precursor cell, the haemopoietic colony-forming unit (CFU), is known to travel in the same direction at the same time (Barnes and Loutit, 1967). It remains to be proved whether the two types of precursor are identical or not: there is evidence that cells derived from CFU can colonize lymphoid organs, but not that they actually make antibody (Wu, Till, Siminovitch and McCulloch, 1968).

In the NZB liver there appear to be more PFC precursors for SRBC than for chicken RBC, while in the BALB/c the numbers are similar. It is hard to escape the conclusion that the PFC precursors carry specific information as to what kind of PFC they may engender. Whether such information is present in all the cells, in the form of different numbers of base sequences coding for SRBC-antibodies, or whether there are different numbers of cells with exclusively SRBC-antibody information, cannot be deduced from the present data. But it does seem possible to rule out a third alternative, namely that liver PFC precursors are totally neutral, responding impartially to the instructions of a thymus cell.

(2) The *thymus PFC inducer*, on the other hand, shows no predilection for SRBC in the NZB, but may perhaps be generally less numerous, or less effective, in the C57BL. This recalls the results of thymus grafting in the newborn, where NZB and BALB/c thymuses were equally good, and C57BL less good, at restoring the PFC response of thymectomized BALB/c mice (Playfair, 1968). Whether a thymus graft and an intravenous injection of thymus cells (which are mostly lymphocytes when prepared by teasing) promote immunological activity by the same mechanism is not yet clear; possibly longer-term humoral properties may be lacking in the cell suspensions, but cells are known to leave the thymus and colonize peripheral lymphoid tissue (Weissman, 1967), so the intravenous injection of thymus cells may not be altogether unphysiological. Perhaps the overall development of

immune responsiveness depends on both kinds of thymic activity, while the specific types of antibody that can be made are decided by genetic information in the liver cells. If it were to be confirmed that genetic information in the liver cells is indeed absent from thymus cells, the implication would be that the genetic diversity arises in the liver at a late stage, since both liver and thymus 'stem cells' are ultimately derived from the same tissue—the embryonic yolk-sac (Moore and Owen, 1967). It by no means follows that thymus cells are not involved in the recognition of antigen; on the contrary, the successful transfer of tolerance by a thymus graft (Isakovic, Smith and Waksman, 1965) suggests that they are concerned in at least the initial detection of foreignness. The above concepts are in agreement with the recent suggestion of Burnet (1968) that immune recognition essentially involves cells of thymus origin—the 'cellular response'—and that the humoral antibody-forming system represents a phylogenetically later addition, incorporating refinements in specificity.

It should be stressed that the actual counts reported here are of antibody-forming cells—not precursors, the enumeration of which is more tedious and less accurate. However, it has been estimated that a 'spleen PFC precursor' produces an average of 20–100 SRBC PFC (Kennedy *et al.*, 1965), and it is also known that about 5–10 per cent of injected spleen or marrow cells reach the irradiated host spleen (Claman *et al.*, 1966). From these figures one can calculate that the 11,000 'new' PFC produced by syngeneic liver and thymus in the NZB might represent at least 1100, and at most 11,000 PFC precursors. If the spleen PFC precursor is derived from the liver PFC precursor without further division, this would also be the number of liver PFC precursors contained in the $15 \times 10^6$ cells injected—a proportion of between 1 : 800 and 1 : 8000 of all nucleated newborn liver cells.

*The role of histocompatibility in cell transfer experiments*

The advantage of using $F_1$ hybrid mice as the irradiated hosts to cells from different inbred strains is that all the variable ingredients of the immune response can be standardized, apart from the particular cells to be tested. Moreover, hybrid mice will survive much higher doses of X-rays. Nevertheless, it is important to know how far the inevitable histocompatibility differences influence the results.

Relevant observations have been made by Chaperon and Claman (1967) who found that both spleen and mixtures of thymus and marrow gave better PFC responses in irradiated syngeneic than in $F_1$ or allogeneic hosts. On the other hand, Lawrence and Simonsen (1967) found that parental spleen cells sometimes gave better PFC responses in $F_1$ than in syngeneic hosts, but only when SRBC were injected early after irradiation; they postulated an initial non-specific stimulation of *all* responses, followed later by a depression of all but the graft-*versus*-host (GVH) response.

In our (NZB × C57BL)$F_1$ hybrids (Fig. 3), syngeneic thymus plus liver (or marrow) gave an average of about 1300 PFC. Replacement of *either* thymus or liver by NZB tissue raised the PFC about three-fold, so it might have been predicted that the combination of NZB liver *and* thymus would give a nine-fold increase, or some 12,000 PFC—which is precisely the figure that NZB liver and thymus had given in NZB hosts (Fig. 1). In the event, the average PFC count was only 1400. That is to say, NZB liver and thymus, individually better than their $F_1$ counterparts, lost their superiority when combined in the $F_1$ host. In view of the findings of Chaperon and Claman (1967), it is tempting to explain this drop in PFC response as the reflection of an increase in GVH reaction, such as might indeed be

expected of 'competent' NZB cells in an $F_1$ environment. If this is the true explanation, it implies that GVH 'competence' can only be achieved when both the thymus and the liver cells are of NZB origin; substitution of $F_1$ thymus or liver (which are, for the purposes of the GVH reaction, tolerant cells) decreases or abolishes the GVH response, allowing a higher SRBC response. Experiments are under way to test this hypothesis directly.

*Autoantibodies and the NZB strain.*

A unique feature of NZB mice is the development of autoantibodies against autologous RBC (Bielschowsky, Helyer and Howie, 1959). Helyer and Howie (1963) have shown, and we have confirmed, that these autoantibodies can be induced in CBA mice if they are thymectomized and grafted with a newborn NZB thymus. We have also found (Allman, Playfair and Roitt, unpublished) that a neonatal injection of bone-marrow from young NZB mice can have the same effect. If the production of such autoantibodies is to be regarded as a loss of self-tolerance, these results imply that faulty recognition of self-antigens by *either* thymus or marrow is enough to provoke autoantibody formation, which at first sight clashes with the hypothesis just stated—namely that *both* cell types must be 'competent'. However, in the CBA mice, several months elapse before the autoantibodies are detected, which perhaps allows time for marrow cells to colonize the thymus, as they are known to do (Moore and Owen, 1967). The transfer with thymus alone is harder to explain, unless it is postulated that CBA marrow is already unusual in containing the information to make RBC autoantibodies, in rather the same way that NZB liver contains unusual amounts of SRBC-antibody information.

*The C57BL strain*

A disappointment of the present experiments was that the C57BL liver cells could not be properly evaluated, owing to their peculiar reluctance to grow in $F_1$ hosts. This effect has been observed on many occasions (Popp, 1961; Cudkowicz and Stimpfling, 1964), and has been shown, by an ingenious experiment using $F_1$ and $F_2$ hybrids (Popp and Cudkowicz, 1965) to be linked to the H-$2^b$ allele. The inhibition is not a straightforward immunological one, since C57BL cells grow better in totally allogeneic mice than in the semi-syngeneic $F_1$ hybrid (Cudkowicz and Stimpfling, 1964).

It is interesting to wonder whether there is any connection between this effect and the unusually weak humoral antibody potential of neonatal C57BL mice, reported here and previously (Playfair, 1968). Inability to make antibody against transplantation antigens, or lack of some equivalent surface feature, might allow closer contact with foreign cells, provided they in turn lacked anti-C57BL antibody, as would be the case with $F_1$ hybrid, but not with allogeneic cells. Close contact with foreign cells, under the name of 'allogeneic inhibition', has been shown to induce mutual cell death (Möller, 1965). Direct evidence on the role of antibody in GVH reactions has been conflicting; in rats, resistance against GVH is associated with a serum factor, probably isoantibody (Field, Cauchi and Gibbs, 1967), while in mice the addition to parental spleen cells of anti-host antibody can cause either more or less severe GVH disease (Batchelor and Howard, 1965). That cell death alone may not explain the deficient C57BL repopulation is suggested by the finding of surviving C57BL cells months after an apparently unsuccessful transfer (Popp, 1961). Clearly this intriguing anomaly needs further study.

Meanwhile it is planned to repeat the above experiments with the C57BL/Ks strain, which carries the H-$2^d$ allele and, therefore, may have the double advantage of closer

histocompatibility with the NZB and BALB/c (both also H-$2^d$) and freedom from the 'C57BL effect'.

Another question concerning the C57BL strain arises from the finding of so few PFC precursors and inducers in neonatal liver and thymus, since: (1) the PFC response in C57BL mice does eventually reach normal levels (by about 3 months), (2) liver PFC precursors decline in numbers after the 2nd day, and (3) the adult PFC response increases normally in the absence of the thymus, provided thymectomy is delayed until after the 1st week of life (Playfair, 1968). Therefore, either the adult response represents a large expansion of a small 'starting' population of precursor cells by some non-thymic influence, or there must be a source of PFC precursors other than the foetal liver. In the search for non-thymic stimulators of humoral antibody-forming tissue one naturally thinks of the gut-associated lymphoid organs, which have been put forward as the mammalian equivalent of the avian bursa (Cooper, Perey, McKneally, Gabrielsen, Sutherland and Good, 1966). So far I can only report that Peyer's patch cells, transferred to irradiated mice, do not give rise to any PFC either alone or in combination with bone-marrow. In this respect they are certainly different from other lymphocytes, but do not seem to share the inducing function of thymocytes, at least where haemolytic SRBC antibody is concerned.

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