FURTHER INVESTIGATIONS OF ANTIGENIC STRENGTH BY THE MIXED SPLEEN CELL REACTION

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The mixed leucocyte reaction has been proposed as a useful technique in the study of antigenic strength and thus of value in the selection of donors for transplantation. A positive correlation between the strength of the reaction and the genetic disparity of the donors has been claimed (Bain and Lowenstein, 1964; Moynihan, Jackson and Hardy, 1965; Rubin, Stenzel, Hirschhorn and Bach, 1964). However, a less clear correlation of this nature was found by other workers (Elves and Israels, 1965; Eijsvoogel, 1967).

Previous studies using the mixed spleen cell reaction in inbred mice did not show a positive correlation between the known histocompatibility differences and the strength of this reaction (Festenstein, 1966). Secondary inhibition followed the peak of radioactive label incorporation, and was most marked in the strong H-2 different mixtures. Contrary to expectations, the highest peaks were found, though a little delayed, in the weak non-H-2 mixture studied. It was suggested that this might be due to allogeneic inhibition. If Fl hybrid spleen cells are mixed with spleen cells derived from the parent strains, one would expect an immunological one-way reaction. This paper describes the mixed spleen cell reaction between hybrid and parent strain inbred mice in strong H-2 different combinations and weak non-H-2 different combinations. In addition the results of the mixed spleen cell reaction between male and female mice of the same Fl hybrid strain are reported. These experiments were carried out to determine whether the single weak sex difference can be detected in this system.

Methods

The method of culture is that described earlier. Suspensions of living spleen cells from 2-3 months old female mice of strains CBA, DBA/2, Balb/c, CBA × DBA/2Fl, Balb/c × DBA/2Fl, were cultured separately ('background') and in paired mixtures. The 'strong' H-2 different mixtures were CBA plus CBA × DBA/2Fl, and DBA/2 plus CBA × DBA/2Fl, the 'weak' non-H-2 mixtures were Balb/c plus Balb/c × DBA/2Fl and DBA/2 plus Balb/c × DBA/2Fl. For the male-female mixed spleen cultures, C57BL × CBA Fl hybrids were used. The intensity of the reaction was measured daily by the uptake of $^{14}$C thymidine for a total time course of 5 days.

Foetal calf serum (Difco) and colostrum free newborn calf serum (Buroughs Wellcome) were used to concentrations of 7% and 10%, respectively. Part of the culture medium was changed on the 3rd day by replacing 1 ml with 1 ml of Eagles' MEM containing 5% serum.

Results

Mixed cultures of equal numbers of spleen cells of CBA plus CBA × DBA/2Fl (C + C × D), DBA/2 plus CBA × DBA/2Fl (D + C × D), Balb/c plus Balb/c × DBA/2Fl (B + B × D) and DBA/2 plus Balb/c × DBA/2Fl (D + B × D) as well as their 'background'
controls were all set up simultaneously. The 'peak' or day of maximum stimulation was again taken as that point where the difference between the uptake of label by the mixed culture and its respective background was maximal. The results of one of 2 experiments carried out under as closely similar conditions are shown in the figures. As in previous experiments of this nature, the relative extent of label incorporation in the mixed cultures varied within the experiments according to the day of observation and a consistent picture emerged when the various strain combinations were ranked according to peak incorporation attained over the whole time course (Table I). The highest peak was given by C + C × D (strong) followed by B + B × D (weak), D + C × D (strong) and finally D + B × D (weak).

![Spleen cells from CBA × DBA/2 and Balb/c × DBA/2 F1 hybrids and their parent strains cultured alone and in paired mixtures.](image)

Spleen cells from C57 × CBA F1 hybrid males and females were similarly set up in culture in paired mixtures with background controls. The results of three such experiments are shown in Table II. Increased uptake of radioactive label in the mixtures was not observed in any of the experiments over a 3.5-4.5 day time course.

**Discussion**

The failure of previous experiments to show a correlation between the intensity of the mixed spleen cell reaction and the genetic differences of the donor mice was thought to be due to the insufficiently controlled result of 2 opposite processes which are both rooted in the existing histocompatibility difference—namely primary immune response counteracted by alloimmune inhibition. The results of the reported experiments using hybrid-parent mixtures where 'one-way' stimulation only is expected to be operative, still do not give results which can be accurately correlated with histocompatibility differences; further circumstantial evidence is thus provided in favour of simultaneous inhibition of DNA synthesis induced by contact of allogeneic spleen cells. The possibility therefore of obtaining meaningful results in
### TABLE I

Comparison of peaks* $^{14}$C-thymidine incorporation in mixed cultures of CBA × DBA/2 and BALB/C × DBA/2 F1 hybrid mouse spleen cells with their parent strain spleens over a 5-day time course.

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<tr>
<td>518</td>
<td>C × C × D</td>
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<td>2124</td>
<td>D × C × D</td>
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<td>3.5</td>
<td>B × B × D</td>
<td>795</td>
<td>3.5</td>
<td>608</td>
<td>D × B × D</td>
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* Day of peak stimulation is that day when the C.P.M./10^6 cells of mixed cultures show the greatest difference as compared with the C.P.M./10^6 cells of their respective 'backgrounds', e.g. in the case of C × C × D = mixture of CBA + CBA × DBA/2 and the 'background' the mean of CBA + CBA × DBA/2. The 'background' values have been subtracted in each case.
FURTHER INVESTIGATIONS OF ANTIGENIC STRENGTH

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Day 1.5</th>
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<td></td>
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<td>Female</td>
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<tr>
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<td>793</td>
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<td>994</td>
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* Results given as mean counts of triplicate culture tubes per minute/10^6 cells measured during 5 days of culture in 16 hour pulse periods.
† Background expresses the mean of the respective controls.

terms of histocompatibility differences with mixed cultures of cells from different individuals, as currently tested, even if a true one-way immunological reaction can be obtained, appears doubtful.

The lack of response between cells of different sexes indicates that this reaction is not sensitive enough to detect such weak differences. These results confirm Dutton's (1966) findings with male and female mixed cultures although he did not study this reaction beyond 48 hours.

ACKNOWLEDGMENT

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REFERENCES


EIJVOGEL, V. P., SCHELLEKENS, P. T. A. and ENGELFRIEDT, C. P.: *This Volume*, p. 198.


DISCUSSION

The Chairman: Thank you very much.
Are there any questions?

Cepellini (Turin): The data that have been presented are very interesting because they probably emphasize what has been underlined by Elstrom and Muller, this phenomenon of the syngenic preference.

I want to point out however one problem, and maybe I direct my answer to Dr. Friedman. He asked if a genetic theory would differentiate between parents and siblings.

In the genetic theory in general, parents and siblings have the same value but in the case of incompatibility, where there is a heterozygote that is incompatible for the donor, only in this case siblings and parents are differentiated.

The fact that the observations show that parents are not as good as siblings rules out the importance for the transplantation of syngenic preference; it shows that really incompatibility is the basis of that. Utilizing some special test like the mixed cultures, it may very well be that big differences have a big effect which has not an immunological basis.

But I want to ask this: has anybody tried to mix F1 with parental lines with sex markers, and see if both cells, both populations are activated.

I suspect that even when there is only one-direction incompatibility, also the second population is activated. But we do not have any clear proof of that.

Festenstein (East Grinstead): I do not think that experiment has been made.

The Chairman: I would like to make a brief comment about Dr. Lejeune’s paper if I may.

That is that dosage, way of administration and preparation of the antigen, make such a difference in whether one gets tolerance, no reaction at all or sometimes immunization.

This is particularly important, of course, for the human problem, because it is uncertain in the animal.

Here, in the July issue of ‘Transplantation’, there are two studies, one of which shows prolongation of grafts by subcutaneous administration of spleen cells, and the other which shows accelerated rejection by the same technique.

Dr. Dausset mentioned that his intravenous injections of cells gave white grafts in humans. We were totally unable to do this with intravenous injections of cells and yet it very easily with intradermal injections.

Furthermore, Dr. Rappaport has shown that the preparation of the antigen, whether it is sonically disrupted, or whether it is frozen, will make a difference in whether one gets sensitivity or tolerance.

So that before taking any of these studies and applying them to man, in whom the possibility exists that you might actually sensitize him to the graft, I think we have to clarify this field very obviously.

Dr. Dossettor, I think, has one comment which may end this particular session.

Dossettor (Montreal): I would like to show a slide to give some clinical basis to the supposition that there are major and minor groups involved in tissue incompatibilities. It shows the result of transplanting both kidneys from the same donor to two randomly selected recipients.
DISCUSSION

We have done this seven times and on five occasions the kidneys survived long enough to permit an immunological interpretation of their fate. On three occasions the kidneys behaved differently in the two recipients.

However, on the two remaining occasions the pairs of randomly selected recipients have behaved identically. On the first occasion both recipients showed a massive rejection reaction indicated by a sharp rise in blood urea and serum creatinine at two and three weeks respectively after the transplant.

On the second occasion both recipients of a pair of kidneys from the same donor showed no rejection reaction at any time, although the same immunosuppressive medication was used as in the previous pair. Presumably, therefore, in these two latter recipients the kidney tissue did not have a major incompatibility antigen.

Because of non-immunological complications a second kidney was placed in one of these latter patients. This was also randomly selected and it underwent irreversible rejection. This would suggest that, contrary to the opinion of Dr. Hume, the fate of the second kidney is also determined by immunological factors and it will not necessarily be better accepted.