A PRELIMINARY STUDY OF IMMUNOLOGICAL TOLERANCE IN HUMAN NEWBORN BABY BASED ON MIXED LEUCOCYTE CULTURES

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Bain *et al.* (1964) reported that mixed cultures of leucocytes from two genetically dissimilar donors resulted in transformation showing mitosis, and that the mixed lymphocyte reaction was markedly depressed in mixed cells obtained from either monozygotic twins or from inbred strains of rats. This finding has inaugurated a new phase of study of immunological tolerance with the use of lymphocytic function.

Results obtained with human subjects by Bain *et al.* (1964), Rubin *et al.* (1964), Bach and Voynow (1966), and Bach and Amos (1967) have indicated that the mixed lymphocyte reaction reflected differences in histocompatibility between reacting cells. Nowadays, a good deal of information is available indicating that the degree of the mixed leucocytes culture reaction between two different groups was illustrated on the blastic transformation which was measured by incorporation of ^3H - or ^{14}C -thymidine into cells synthesizing deoxyribonucleic acid (DNA). It has been suggested that the mixed leucocyte cultures appear to be an effective method for measuring the degree of the immune reactivity.

The present study was undertaken to evaluate immunological tolerance in newborn babies by using the mixed leucocyte culture and autoradiographic techniques.

MATERIALS AND METHODS

The subjects used in the present study were mothers and their newborn babies who were all unrelated to one another.

Mixed cultures were obtained with leucocytes from 17 combinations of two unrelated newborn babies (N/N group), from 25 combinations of a newborn baby and an unrelated mother (N/M group), and from 101 combinations of two unrelated mothers as a control group (Ohama and Kadotani 1971).

Venous blood, in case of newborn babies from umbilical cords, was drawn, 10 ml from each individual, into a heparinized syringe and allowed to stand for 1-2 hours at room temperature. Supernatant plasma obtained from each blood, containing leucocytes and other blood elements, 0.5 ml in volume, from each combination was then mixed, added to 3.0 ml of TC 199 medium, and cultured at 37°C. After five days of

incubation, ^3H -thymidine (specific activity 5.0 Ci/mmol) was added to the medium at a concentration of $1.5 \mu\text{Ci/ml}$ and the culture was incubated for 3 hours and followed by treatment with colchicine (10^{-7} M) for another 3 hours. The cells were harvested, treated with hypotonic solution, fixed with acetic alcohol, and air-dried. The slides were coated with Sakura NR-M₂ liquid solution. After 10 days of exposure at 4°C , the slides were developed with Conidol-X for 5 minutes at 20°C , fixed, washed, and stained with 10 percent Giemsa solution. 1000 mononuclear cells in interphase were counted under the microscope and the number of labeled cells were noted. In each case uncoated slides were stained with Giemsa for karyotype analysis.

RESULTS

The results of the present investigations are summarized as follows: the mean percentage of labeled cells and standard deviations were 4.3 ± 4.6 for the N/N group, 3.5 ± 4.1 for the N/M group and 6.6 ± 5.5 for control group (Table 1). Among the three groups the mean percentage of labeled cells in the N/M group was significantly lower than in the control group ($p < 0.05$). However, there were no significant differences in the mean percentage between the N/N group and the N/M group, and between the N/N group and the control group.

Table 1. The mean percentage of labelled cells and standard deviations

Combination	No. of combination	Labelled cells (%)
Newborn/Newborn (N/N)	17	4.3 ± 4.6
Newborn/Adult (N/M)	25	3.5 ± 4.1
Adult/Adult (control)	101	6.6 ± 5.5

DISCUSSION

Owen (1946), studying blood groups in cattle, has provided an important information that twin cattle which were not identical twin but were dizygotic, i.e., shared in the same placental circulation in the uterus and exchanged a large proportion of their red and white cells during fetal life, could sometimes be shown later to possess circulating red cells not only belonging to their own blood group but also to the different groups of their twin. Afterwards, Medawar and Woodruff (1958), studying with some closely inbred and genetically almost uniform mouse strains, reported that when an "A" mouse baby was injected with living cells from a "B" mouse embryo, the recipient mouse developed normally and skin graft from "B" mouse was taken and remained healthy. The above findings seem to suggest that immunological tolerance, i.e., the ability of the animal to allow not-self cells to live develops and antibodies are not produced during development.

The degree of mixed leucocyte culture reaction from two unrelated embryos or newborn, when viewed from immunological tolerance and from mutual reaction of lymphocytes obtained from two different individuals, may be lower than that of newborn-adult combination. Also, the degree the reaction from newborn-adult combination may be lower than that from adult-adult combination.

In the present study, the degree of the mixed leucocyte culture reaction from newborn-adult combinations was significantly lower than that from adult-adult combinations (control group). The degree of the mixed culture reaction from newborn-newborn combinations seemed to be lower than that of control group and was nearly equal to that from the newborn-adult combinations, although not statistically significant.

Wilson (1965) showed that the destruction of homologous target cells by sensitized lymphoid cells occurred in a two-step-process: first, the attacking lymphocytes firmly adhered to the target cells having an immunologic specificity; and the cytotoxic effect of these lymphocytes generally occurred. Leventhal and Oppenheim (1969) showed that cell density was critical in obtaining a response to antigens. The cell density increases either with the increases in the number of cells in a vial of standard size or with the decrease in the surface area available for the cells to settle down. It is well known that the density of leucocytes in blood plasma of newborn babies is higher than that of adults. Owing to the above circumstances, it is considered that, although the immunological tolerance is truly lower in newborn babies, the mixed leucocyte reaction of newborn-newborn combination was relatively higher than that of the newborn-adult combination.

It is suggested that the investigation of embryo immunity should be carried out in future with the unidirectional system and the constant cell density.

SUMMARY

The degree of mixed leucocyte reaction from newborn-adult combination was significantly lower than that from the adult-adult combination. Although not statistically significant, the mixed leucocyte reaction from newborn-newborn combination was lower than that of adult-adult combination, and nearly equal to that from newborn-adult combination.

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