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Impaired Capacity of the Macrophages of Newborn Mice for T-Lymphocyte Activation by Phytohemagglutinin

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The level of collaboration in phytohemagglutinin (PHA) induced lymphoblastic response by the macrophage of neonatal mice was studied. The results show a diminished response to the mitogen (PHA) when lymphocytes from the spleen of syngeneic adult mice were incubated in the presence of macrophages from neonatal mice as compared to those obtained from cultures performed in the presence of macrophages from adult mice.

In both cases the highest response corresponded to the lowest dose of PHA and the lowest amount of macrophages used.

There are many immunological differences between newborn and adult animals. These differences have traditionally been attributed to the special characteristic of B and T-lymphocytes (1, 4) which appear at the time of birth and may even last up to the third or fourth week; e.g. tolerance to some foreign antigens which may be easily induced in this early stage of life (6, 7).

Recent data obtained from adult mice (2, 8, 11-14) have demonstrated that macrophages are necessary accesory cells for T-cell activation by mitogens and antigens in *in vitro* cells culture assays (15, 17, 18).

However, there is very little information available as to the role the macrophage plays in newborn immune response. This paper studies the development of T-cell-macrophage collaboration processes in neonatal mice.

Materials and Methods

CBA/H mice from the London Hospital Medical College and bred in our laboratory were used. Mice between 2 and 3 months old were considered adult and those between 3 and 5 days old newborn.

Culture medium. All the cultures were grown in RPMI 1640 (Difco) containing ampicillin (100 μg/l), cloxacillin (100 μg/l) and gentamycin (5 μg/l), as well as AB^+ human normal serum, previously heat-inactivated to a final concentration of 5 %. The pH was adjusted between 7.2-7.4 with 4.4 % sodium bicarbonate (Wellcome).

Cell preparation. The macrophages were obtained from the peritoneum of mice sacrificed by cervical dislocation. 4 ml of the above culture medium were injected i.p. to the adult and 0.5 ml to the neonatal mice. After massage of the abdomen the enriched cell medium was removed and in both cases the cells were adjusted to three different concentrations: 2×10^5, 10^5 and 2×10^4 per ml.

The lymphocytes were obtained from the spleen of adult mice, also killed by cervical dislocation. Their spleens were removed aseptically, freed of fat, minced with scissors and gently pressed through a mesh screen to produce a single cell suspension. This cellular suspension was centrifuged in a Urograph-Ficoll gradient at a density of 1080 and at 2500 r.p.m. The halo obtained (95 % lymphocytes and 5 % leukocytes and platelets) was resuspended in the medium and washed twice in PBS. These cells were partially purified by passage over a nylon-wool column. The eluted spleen lymphocytes were then further purified by incubation (45 min, 37° C) in a packed nylon-wool adherence column. The non-adherent cells were obtained by slow elution without compressing the wool (9, 10).

Macrophage-lymphocyte culture. Macrophages aliquots of 0.2 ml were placed in a flat bottom «Nunclon» plate. After 2 h of incubation in an atmosphere of 5 % CO_2 at 37° C, the non-adherent cells were removed by several washings with culture medium, leaving behind the attached macrophage monolayer; 0.2 ml of lymphocyte suspension (10^6/ml) was then added to these macrophages and incubated 48 h.

When this period was up 0.16 μCi thymidine tritiated (^{3}H-TdR) was added to each well and incubated for another 16 h. The cells were collected in a London Miniharvester, dried and counted in a liquid scintillation counter (LKB) (5).

Adult and newborn macrophage number control. Before performing any experiment, the number of macrophages were adjusted by counting. The number of these cells were controlled during the experiments by measuring them in the washing fluid and by seeing its uptake of sodium chromate ^{51}Cr (CEA). The ^{51}Cr is a specific marker of the number of cells. These results are shown on tables I and II, respectively.

Results

The ^{3}H-TdR uptake by lymphocytes from adult mice cultures with different amounts of macrophages from newborn and adult mice with three different dosis of mitogen (PHA) are shown in figure 1. The degree of stimulation is higher when

Table I. *Macrophages in the washing fluids.*

No. Macrophages/ml	Adults	Neonatals
200×10^3	91 ± 15	123 ± 13
100×10^3	103 ± 33	105 ± 6
20×10^3	78 ± 12	59 ± 9

Table II. *^{51}Cr uptake by peritoneal adherent cells ($\bar{x}$ ± S.D.).*

	c.p.m.	
Macrophages/ml	Adults	Neonatals
200×10^3	17742±1194	14372±1536
100×10^3	12673±2359	12270± 737
20×10^3	7455±1726	8048±1007

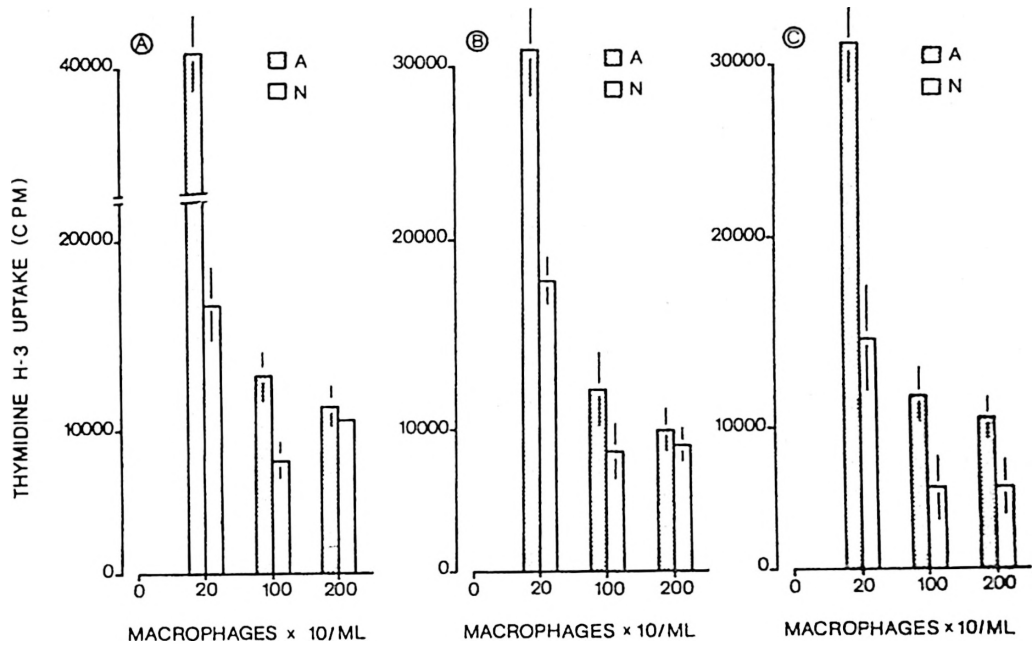

Fig. 1. *Lymphocyte response to 3 different PHA dosis incubated in the presence of different amounts of macrophages taken from adult (dotted columns) or neonatal (empty colums) mice.*

a: 0.07 μg/ml PHA; *b*: 0.14 μg/ml PHA; *c*: 0.28 μg/ml PHA. Each value represents the mean value (cpm) of eight experiments.

the lowest dose of PHA was used and when the lowest amount of adult and newborn macrophages were cultured.

Pure cell cultures (lymphocyte, newborn and adult macrophages) were made at the same time and considered controls. The ^{3}H-TdR uptake was lower than 3,000 c.p.m. in all cases.

Discussion

In order to clarify whether macrophages of neonatal mice are involved in the immunological differences between newborn and adult mice, macrophages from either adult or neonatal animals were incubated with spleen lymphocytes from syngeneic adult mice and stimulated with different doses of phytohaemagglutinin (PHA).

This study found that there is a diminished response by the lymphocytes to PHA when incubated with macrophages from neonatal mice.

The ^{3}H-TdR uptake when neonatal and adult macrophages were incubated separately indicates that the effect observed is not due to a phagocytosis carried out by the macrophages.

In the same way, the ^{3}H-TdR uptake by the lymphocytes incubated alone is not statistically significant when compared to the uptake of this marker in the presence of macrophages taken from either adult or neonatal mice.

The effect observed could be due to the suppressor activity of a contaminant population of lymphocytes from the peritoneum. However, there are no data available which supports this. GRANGBERG *et al.* (3) have recently demonstrated that

newborn suppressor activity exerts most of its influences on the function of the B cells and not on the T-lymphocytes one.

Morever, SNELL and co-workers (16) have also demonstrated that the lymphocyte population that may be affected by T-suppressor lymphocytes corresponds antigenically to B and not T cells.

On the other hand, the mechanism by which the mitogen induces a high level of response when incubated with macrophages from adult mice and not neonatal ones remains uncertain.

LU *et al.* (4) have demonstrated that a correlation exists between the low capacity of the neonatal macrophages to collaborate with lymphocytes to develop an immune response and the small number of Ia^+ macrophage subpopulation.

The results support the idea that macrophages from newborn mice are functionally limited in the process of taking up and handling the mitogen and could present a defect in the presentation of the mitogen with the adequate immunogenicity to the effector lymphocytes.

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Resumen

Se estudia el grado de colaboración de los macrófagos de peritoneo de ratones (CBA/H) recién nacidos en los procesos de activación de los linfocitos T por la fitohemaglutinina (PHA). Los resultados muestran que existe una respuesta inferior cuando los linfocitos del bazo de ratones singénicos adultos son incubados en presencia de macrófagos de recién nacido que cuando estos linfocitos se incuban con macrófagos de ratones adultos. La máxima respuesta se produce tanto con macrófagos de adultos como de recién nacidos, cuando se utiliza la concentración inferior de macrófagos, así como la dosis más baja de PHA.

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