RESPONSE OF MATERNAL IMMUNE CELLS TO IRRADIATION OF MOUSE EMBRYOS

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INTRODUCTION

This work began as an attempt to explain the paradox of pregnancy - the survival and growth of the semi-allogeneic embryo in an immunologically hostile environment. We[3,4] rediscovered the work of Brewer[2] who in 1937 had observed the phagocytosis of maternal blood cells including lymphocytes by cells of the trophoblast. In 1982 and 1983 we reported the tracing of quinacrine labelled maternal leukocytes (WBC) in maternal, placental and embryonic mouse tissues by fluorescence microscopy. We found that cells in the placenta phagocytose labelled WBC, so that after 1-2 hours the labelled nuclear DNA is found as brightly fluorescing particles in the cytoplasm of the phagocytes with no evidence of it in the nuclei. Identical cells were observed in slide preparations of embryos which had been carefully separated from their placentas.

We also found a small population of intact labelled lymphocytes, clearly maternal in origin, in the embryos. This seems to be another paradox- placental phagocytes are observed to be phagocytosing maternal WBC in the placenta and embryo, but there are also free maternal cells in the placenta and embryo. A theoretical explanation is that maternal lymphocytes alloreactive against the embryo will attempt to react with placental cells and in the process be phagocytosed, while other maternal cells will be able to enter the embryo where they could have a surveillance function, removing dead or mutant embryonic cells.

To test this theory we carried out the series of experiments reported here.

MATERIALS AND METHODS

These fall under 3 headings: (1) the quinacrine labelling method referred to above; (2) the use of the histochemical α-naphthyl butyrate esterase (α-NBE) procedure (Markovic, 1987[6]) to identify maternal lymphocytes and monocytes in 11 day-post conception mouse embryos (BALB/c); and (3) the creation of artificial embryonic damage with varying doses of γ-radiation.

(1) Quinacrine labelling. Maternal whole blood (approx. 0.2ml) was labelled with 0.1ml quinacrine solution (9.4 mg/10ml of normal saline). After standing for a few minutes, the blood sample was washed 3X with normal saline to remove quinacrine unbound to the WBC nuclei. The blood was then reinjected into the original donor. Two hours or more later the mouse was sacrificed and various tissues, placentas and embryos

were examined. Embryonic tissues could be dissociated on a microscope slide using the end of another slide, which was then used to make a smear as in making a blood slide.

(2) The α-NBE procedure (modified from Sigma) was used on embryonic tissues: an air dried slide preparation of embryo was fixed for 5 minutes in glutaraldehyde below -10°C, followed by incubation for 1 hour at 37°C with α-NBE reagent. The rinsing step was then repeated, followed by 4 minutes of staining with methylene blue. After a third rinse, the slides could be mounted in aquamount.

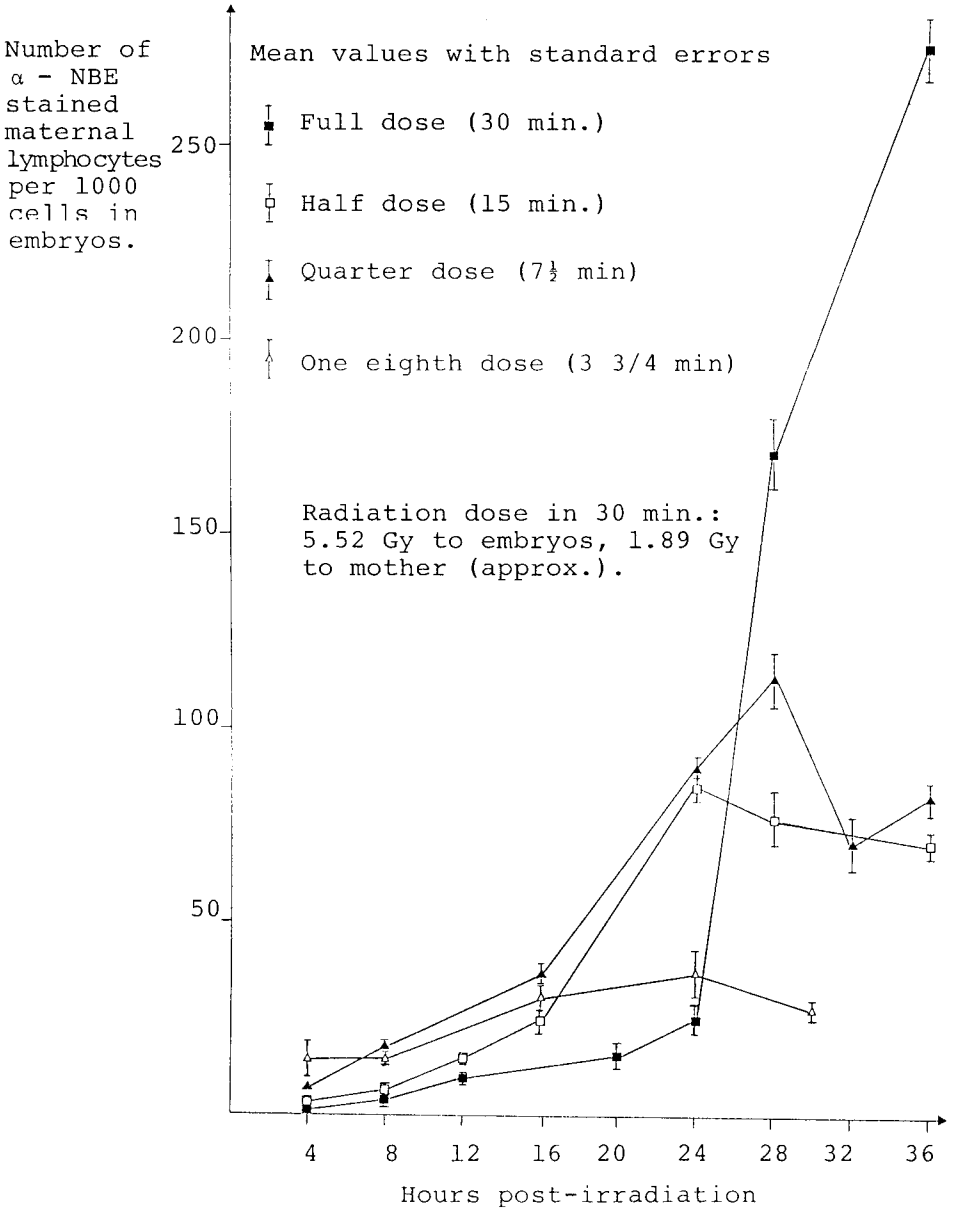
(3) γ-irradiation. A method was devised to lethally irradiate the embryos of a pregnant mouse while delivering a lesser dose to the mother. A Cobalt-60 source of γ-rays was used. The mother was shielded by 30 mm of lead, this being the maximum shielding possible because of the construction of the housing of the source. Two lead radiation chambers were prepared from molten lead cast to fit comfortably into the hydraulically lowered cylinders of the source. A chamber with a diameter just large enough to house a pregnant mouse was moulded lengthwise through each cylinder, from one end almost to the other end. A hole 3 mm in diameter was drilled at the closed end of the cylinder to provide air for the animal while in the chamber. A 10 mm diameter hole was drilled at right angles to the midline of the cylinder at the position where the abdomen of the pregnant mouse, and hence the embryos, would be located and in a position to be aligned with the radiation beam. The mouse was held in position after entering the chamber by inserting a plug of foam plastic. Irradiation initially was for 30 minutes, a time chosen because it was shown to give a lethal dose to all embryos. The radiation dose was determined by Thermoluminescence Dosimetry (TLD) and found to be maximally 5.52 Gy/30 min to the embryos. Most maternal tissues received in that time only 1.89 Gy. Dosages of radiation were subsequently varied by serial 2-fold reductions of radiation time down to a 1/8th dose (3 3/4 minutes, 0.69 Gy to embryos, 0.24 Gy to mother).

RESULTS

(1) Quinacrine labelling. This method is indirect in determining the population of maternal cells in the embryo. About 10% of the maternal blood was labelled in each experiment. An estimate could be made of the number of WBC this entailed. However, the precise division between circulating and tissue populations of WBC could not be ascertained, and more importantly perhaps, the number of maternal WBC phagocytosed while entering the embryo was not known. Thus this was a qualitative rather than quantitative study. It was seen that the numbers of labelled lymphocytes in the embryo increased after irradiation of the pregnant mice (Markovic, 1984[5]).

(2) α-NBE staining. The figure shows the results of examining 205 embryos. The lymphocyte counts represent direct and total counts of maternal cells (the embryo develops its own mature lymphocytes (α-NBE +ve) after day 15 of pregnancy (Bannerman, 1983[1])

Figure: Maternal lymphocyte numbers in embryos after various doses of radiation, and at various times post-irradiation.



DISCUSSION

The preliminary experiments using quinacrine labelling confirmed previous work and expanded our understanding of the processes under observation. (1) The placental phagocytes are very efficient at removing maternal cells. If these maternal cells are those with allogeneic reactivity to the embryo it is easy to understand why embryos are not rejected. (2) A uniform population of labelled maternal lymphocytes was found in every embryo examined.

(3) Placental phagocytes containing fluorescent particles in the cytoplasm were found in every embryo.

The second series of experiments with α-NBE were more informative. Direct counts of numbers of maternal lymphocytes in the embryos could be made. What we had postulated as the mechanism of reabsorption in naturally reabsorbing embryos was clearly demonstrated with the higher levels of radiation. The embryos contained a massive invasion of maternal lymphocytes at 36 hours (> 25% of total cells in the embryo) and the relationship of this value to the normal population of lymphocytes in the embryos could be seen. Lowering the dose of radiation to a level where most embryos would survive showed two things: (1) the maternal influx of lymphocytes occurred earlier with the lower doses, thus negating the objection that lymphocytes were entering the embryos only because of placental damage; (2) the peak values appeared earlier, 24-32 hours after irradiation, than occurred with the lethal dose of irradiation.

CONCLUSION

We have in these experiments a valuable method for the study of irradiation mechanisms as they affect pregnancy. Much more work can be done. The dose-response relationships are clear. Recent experiments have demonstrated comparable results using toxic chemicals, and hence the method may have value as a screening method in toxicology.

The theoretical implications are also significant. The concept can be developed as follows: artificial damage to, or natural death of, embryos leads to a stimulation of some of the non-alloreactive lymphocytes in the embryos. These lymphocytes send messages via lymphokines to the placenta where more maternal leukocytes will enter the embryo. With this influx the embryo will either be purged of damaged cells or will be destroyed and reabsorbed. Thus there is an efficient tissue surveillance mechanism operating in the otherwise immunologically inert embryos.

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