Iranian J. Publ. Hlth,
Summer 1975, Vol. 4, No. 2

SUPPRESSION OF MICE IMMUNE RESPONSE BY ANTIMACROPHAGE SERUM TREATEMENT AND ITS RECONSTITUTION BY MACROPHAGE TRANSPLANTATION

Nahid Mohagheghpour,
Late Kiarash Nafici

ABSTRACT

The immunosuppressive effect of antimacrophage serum (AMS) on the primary immune response of mice to sheep red blood cells (SRBC) was studied. AMS, given before a small dose of antigen, abrogated the immune response. Transplantation of normal, glass-adherent macrophages enabled AMS-treated animals to respond to SRBC, while administration of lymph node lymphocytes did not reverse AMS - induced immunosuppression significanlty.

Section of Viral Diseases, Department of Pathobiology, Graduate School of Public Health and Institute of Public Health Research, University of Teheran, P.O. Box 1310, Teheran, Iran.

This investigation was supported in part by funds from the Public Health Research Project of the Iranian Ministry of Health and Plan Organization.

INTRODUCTION

Macrophages appear to play an important role in the inductive phase of the primpary immune response 1-4. Recently, antimacrophage serum, as well as other immunosuppressive agents 5-14, has been used as a tool to further study the participation of these cells in the immune response. There is general agreement that antimacrophage serum (AMS) is cytotoxic to macrophages and decreases their phagocytic activity; however, agreement concerning the immunosuppressive activity of AMS is lacking. The experiments of Panijel and Cayeux 5. Argyris and Plotkin 6,7 and Isa 8 have indicated that AMS suppresses the immune response of mice to bacteriophage 0X174, sheep red blood cells, and trachoma agent antigen, respectively. On the other hand, Unanue 9, Loewi *et al.* 10 and Gallily 11 reported that AMS is ineffective in suppressing the immune response of mice to keyhole limpet hemocyanin, sheep red blood cells, and Shigella.

We present evidence that AMS, raised against peritoneal macrophages which were cultured on glass, impairs the immune response of mice to a small dose of sheep red blood cells and that transplantation of normal macrophages restores the immune response of such AMS-treated animals.

Materials and methods. Antimacrophage serum (AMS) was prepared by injecting New Zealand rabbits twice with glass-adherent peritoneal exudate cells (PEC) obtained from adult white Swiss male mice. Cell donors were injected four days earlier with 2 ml of 10% peptone (Difco Laboratories, Detroit 1, Michigan). Peritoneal cells were withdrawn from the mice and washed three times with Hanks' balanced salt solution (BSS) and resuspended in BSS. These cells were cultured overnight in a medium consisting of 10% fetal calf serum and 90% BSS. The medium contained 100 units of penicillin and 100 μg of streptomycin per ml. The glass-adherent cells, which by morphological criteria comprised about 95-98% macrophages, were used to immunize the rabbits. Each rabbit received intravenously and in the footpads a total of 10^8 macrophages. The two injections were spaced 14 days apart and the animals were beld on the 21st day. Serum samples were pooled, decomplemented 56°C for 20 min), and Forssman antibodies were removed by absorption with freshly prepared packed sheep red blood cells (SRBC). Serum was similarly absorbed with packed mouse red blood cells (MRBC). Normal rabbit serum (NRS) was obtained by bleeding the rabbits before the macrophage injections, and was treated in the same manner as AMS to serve as a control. The sera were absorbed at room temperature for 1-2 hr, then filtered and stored at - 20° C. No hemagglutinins were detected after the absorption. The antimacrophage potency of AMS and NRS was tested by thier capacity to agglutinate the glass-adherent macrophages and to destroy these cells in the presence of guinea pig complement. PEC (5 X 10^6 cells per tube) were incubated with 0.2 ml of varying dilutions of serum for 1 hr at 37 C and then guinea pig complement (GPC) (Microbiological Associates, Bethesda, Maryland) was added to make a final concentration of 5%. After incubation for 30 min the viability of the cells was determined by exposing them to 0.4% erythrosin B.

AMS and NRS were tested *in vivo* by their capacity to alter the mice immune response to SRBC. Sheep red blood cells were collected in Alservers' and stored at 4 C. The cells were washed 3 times with phosphate-buffered saline, PH 7.2, and the cell concentration was determined spectrophotometrically, immediately before being used. Mice received 5 daily intraperitoneal (i.p.) injections of either 0.1 ml of AMS or NRS. On day 6, each group of mice received 0.5 ml of SRBC, i.p., containing either 10^9 or 5 X 10^7 cells. Control mice were injected with SRBC only. Four days after the antigen adminisration, the mice were sacrificed by cervical dislocation and the number of rosette-forming cells (RFC) in their spleens was determined according to the method of Laskov 15. Only cells completely surrounded by SRBC were counted as rosettes.

The ability of normal PEC or lymph node lymphocytes to restore the im-

mune response of AMS- immunosuppressed mice was studied by passive transfer of these cells. Mice received five daily injections of AMS as described above. On dap, 6, each animal was injected, i.p., with 2 X 10^7 glass-adherent macrophages or lymph node lymphocytes along with 5 X 10^7 SRBC. Again the response of these animals to SRBC was measured using RFC assay.

Results. 1. *In vitro* antimacrophage activity of AMS : Antimacrophage serum up to 1 :4000 dilutions agglutinated the PEC. Agglutination was visible grossly and microscopically. After incubation with AMS and GPC, 65-88% of the cells were destroyed. When incubated with NRS and GPC, the number of cells was reduced by only 10%. The specificity of AMS was determined by its lack of cytotoxicity for lymph node lymphocytes in the presence of GPC.

2. *In vivo* action of AMS : When AMS was given in the dosage indicated, it proved to be immpnosuppressive in mice. As is shown in Table 1, treatment with this antiserum prior to the i.p. injection of 5 X 10^7 SRBC was associated with 83% reduction in the number of RFC, as compared with the untreated animals. Adminisration of NRS resulted in only 23% reduction.

Immunosuppression could be demonstrated only at a low antigenic stimulation (5 X 10^7 SRBC). When the antigen dose was increased 20-fold (10^9 SRBC), there was no significant difference between the response of AMS-treated animals and the normal controls. The number of RFC per 10^6 splenic lymphocytes of AMS-treated animals which received 10^9 SRBC was 2.9 X 10^3 This is not significantly different from 44. X 10^3 RFC in the control mice. This observation is in agreement with those of Barth 16 and Argyris and Plotkin 7. They, too, found that the immunosuppressive effect of ALS and AMS is antigen-dose dependent.

3. Reconstitution of the immune response with macrophages : To determine whether the immunosuppressive activity of AMS is the result of a depression in the macrophage function, cellular reconstitution studies were employed. Transplantation of 2 X 10^7 normal glass-adherent PEC into the AMS-treated animals resulted in the reversal of the immunosuppressive action of the AMS. These PEC restored the immune response of mice to SRBC by 64% (Table 2). In contrast, lymph node lymphocytes gave no reconstitution. The data thus suggests that the immunosuppressive activity of AMS is mediated by the macrophages.

Discussion. The data presented here confirms previous observations 5-8 and provides further evidence that AMS-induced immunosuppression is mediated by the macrophages. Earlier work has indicated that the induction of phage-neutralizing, anti-SRBC, and anti-trachoma antibodies was suppressed by AMS administration 5-8. AMS, used in the present investigation, reduced the response of mice to SRBC. Suppression, however, was achieved only when a small dose of antigen was used. The discrepancy between our findings and those which show 9-11 AMS to be ineffective in suppressing the immune response could be due, in part, to the dose of antigen and the amount of AMS

administered.

Further indications that macrophages are the primary target cells of AMS have come from reconstitution experiments which demonstrated that normal, glass-adherent PEC transplanted into AMS-treated mice restored the immune response of such animals, while lymph node lymphocytes did not. Patterson *et al.* 12 have also reported that suppression induced by ALS was restored by transplantation of normal macrophages. They suggested, therefore, that ALS-induced immunosuppression, like that of drugs 14,17, may be the result of an alteration of macrophage function.

Data presented by Unanue 9, Argyris and Plotkin 6, and Loewi *et al.* 10 indicates that AMS administration impairs phagocytic activity of macrophages, and that presented by Shortman and Palmer 18 demonstrates that macrophages produce and release natigenic material from SRBC and that this released material, "processed antigen", upon interaction with lymphocytes, initiates the immune response. In view of these findings, which strongly imply the requirement of macrophages in the induction of the primary immune response to SRBC, the most straight-forward explanation of the AMS-induced impairment of the immunologic function of macrophages would be an alteration in the ability of these cells to take up or "process" antigen.

REFERENCES

1. M.J. FISHMAN and F.L. ADLER, J. Exp. Med. 117, 595 (1963).
2. N.P. FRIEDMAN, A.B. STAVITSKY and J.M. SOLOMON, Science 49, 1106 (1965).
3. P.C. FREI, B. BENACERAF and G.J. THORBECKE, Proc. Nat. Acad. Sci. U.S.A. 53, 20 (1965).
4. D.E. MOSIER, Science 158, 1573 (1967).
5. P. PANIJEL and P. CAYEUX, Immunology 14, 769 (1968).
6. B.F. ARGYRIS and D.H. PLOTKIN, J. Immunol. 103, 372 (1969).
7. B.F. ARGYRIS and D.H. PLOTKIN, Clin. exp. Immunot. 7, 551 (1970).
8. A.M. ISA, J. Immunol. 107, 595 (1971).
9. E.R. UNANUE, Nature 218, 36 (1968).
10. G. LOEWI, A. TEMPLE, A.P.P. NIND and M. AXELRAD, Immunology 16, 99 (1969).
11. R. GALLILY, Clin. exp. Immpnol. 9, 381 (1971).
12. J.T. PATTERSON, J.C. PISON and N.R. DI LUZIO, Proc. Soc. Exp. Biol. Med. 135, 831 (1970).
13. M. FELDMAN and R. GALLILY, *Cold Spring Harbor Sym. Quan. Biol* 32, 395 (1967).
14. A.W. FRISCH and B.J. WILSON, Proc. Exp. Biol. Med. 132, 43 (1969).
15. R. LASKOV, Nature 219, 973 (1968).
16. R.F. BARTH, J. Immunol. 103, 648 (1969).

17. R.J. SHARBAUGH and J.B. GROGAN, J. Bacterial. 100, 117 (1969).
18. K. SHORTMAN and J. PALMER, Cell. Immunol. 2, 399 (1971).

Table I. Effect of AMS on mouse anti-SRBC response as compared with untreated and NRS-treated controls

Treatment	Mo . of Mice	Mean No . RFC/1 x 10^6 Spleen cells $\pm$ Standard error
None	11	3 . 6 x 10^3 $\pm$ 0 . 77
AMS	8	0 . 6 x 10^3 $\pm$ 0 . 03
NRS	4	2 . 8 x 10^3 $\pm$ 0 . 21

Animals received 5 X 10^7 SRBC .

Table II. Effect of PEC transplantation on the reconstitution of the immune response of AMS-treated mice

Trearment	No . of Mice	Mean No . RFC/1 X 10^6 Spleen cells $\pm$ Standard error
None	11	3 . 6 x 10^3 $\pm$ 0 . 77
AMS	8	0 . 6 x 10^3 $\pm$ 0 . 03
AMS + PEC	2	2 . 3 x 10^3 $\pm$ 0 . 37
AMS + lymph node lymphocytes	5	0 . 8 x 10^3 $\pm$ 0 . 24

Animals received 5x 10^7 SRBC .