INTERNATIONAL JOURNAL OF LEPROSY Volume 44, Numbers 1 & 2
Printed in the U.S.A.

The Selective Suppression of an Antibody Response to a Given Antigen

T. Matuhasi, M. Usui and M. Minami

Department of Allergology, Institute of Medical Science
University of Tokyo, Tokyo, Japan

The manipulation of an immune response is one of the most important problems in the field of immunology and many attempts to suppress the immune response to a given antigen have been made. We also have tried experiments on the specific or selective immunosuppression by the use of oil droplets containing an antigen together with an immunosuppressant as reported previously (1). Our working hypothesis is as follows: The antigen-recognizing cells which bear receptors for an antigen would be exposed to injurious action of an immunosuppressive drug, if the antigen could carry the drug with itself at the stage of antigen recognition. Consequently, the subsequent immunologic cell to cell cooperation would not be stimulated and poor antibody formation to the antigen could be expected. The method and some experimental bases for the hypothesis are briefly described in the present paper.

MATERIALS AND METHODS

The preparation of the suspension of oil droplets incorporating antigen and/or immunosuppressant was carried out as reported previously (1). One tenth milliliter of PBS containing both antigen and immunosuppressant or containing antigen alone at the desired concentration was added to 0.5 ml of the oil mixture consisting of 85 ml of sesame oil, 15 ml of Span 85 and 4 g of aluminum monostearate. The mixture was sonicated at 20KHz in ice water until a water-in-oil emulsion was obtained. To the emulsion, 1.4 ml of 2% Tween 80 in PBS was added dropwise, with constant sonication at 20KHz in ice water. The sonication was continued until a homogeneous suspension of oil droplets, water-in-oil-in-water, was obtained. It was confirmed microscopically that the resulting oil droplets enclosed smaller vesicles containing the antigen or antigen-immunosuppressant. In the paper, oil droplets containing, for example, BSA and dexamethasone phosphate (DMP) will be abbreviated to BSA+DMP droplets.

A 0.2 ml sample of the suspension of oil droplets was injected into an F_1 hybrid mouse of DDD X Balb/c. At appropriate intervals after the intraperitoneal injection, mice were bled from the tail vein, and all sera were assayed individually by passive hemagglutination using mouse red blood cells coated with the indicated antigen. All antibody titers were represented as the means of n values in $1:10 \times 2^n$ for five mice.

RESULTS

1. The selection suppression of antibody response to human IgG (HGG) or bovine serum albumin (BSA): The oil droplets containing HGG (10 µg/mouse) together with dexamethasone phosphate (DMP, 1 mg/mouse) were intraperitoneally injected into mice simultaneously with the droplets containing BSA (200 µg/mouse) alone. The mice showed only a slight antibody response to HGG, but the

normal response to BSA. Reversely, DMP coexisting with BSA in the same droplets depressed only the antibody response to BSA without affecting the response to HGG. These findings indicate that DMP coexisting with HGG or BSA in the same oil droplets selectively suppresses the response to HGG or BSA (1, 2).

DMP, however, failed to cause any suppression of antibody response to lipopolysaccharide (LPS) from E. coli, a thymus independent antigen.

2. The selective suppression of antibody response to lipopolysaccharide from E. coli (ELPS) or LPS from Pseudomonas aeruginosa (PLPS): The oil droplets containing ELPS (0.1 μg/mouse) together with Daunorubicin (DR, 0.2 mg/mouse) were injected I.P. into mice simultaneously with the droplets containing PLPS (0.1 μg/mouse) alone. The mice showed a significantly reduced antibody titer and a decreased number of hemolytic plaques against ELPS, but the almost normal response against PLPS. Reversely, the mice receiving PLPS+DR droplets plus ELPS droplets showed a depressed anti-PLPS response but a normal anti-ELPS response (3).

3. Suppressive effect of DMP on the antibody response to MON or POL coexisting with DMP in the same oil droplets: Since HGG and BSA have been regarded as T cell dependent antigens (4) and ELPS and PLPS as T cell independent antigens (5), we have assumed that DMP suppresses only T cell dependent antibody response and DR suppresses T cell independent antibody response in our experimental system. To verify this assumption, we performed the following series of experiments using flagellin monomer (MON) and polymer (POL) from Salmonella adelaide as antigen.

Four groups of five mice each were given MON (25 μg/mouse) droplets, MON+DMP droplets and MON+DR droplets, respectively. As shown in Figures 1a, 1b, the antibodies on day 5 were 2-ME sensitive and those after day 10 were composed of both 2-ME sensitive and resistant antibodies in the mice given MON droplets. The mice injected with MON+DR droplets exhibited a similar anti-MON antibody response to that in control mice given MON droplets, but the mice given MON+DMP droplets showed a significantly reduced anti-MON response.

Similar experiments were performed using 10 μg of POL per mouse instead of MON. The patterns of anti-POL responses in these mice were represented in Figures 1c, 1d. Again, DR did not affect the anti-POL response even though it coexists with POL in the same oil droplets, but DMP suppressed the anti-POL response. The reduction of anti-POL antibody formation, however, was not so remarkable as that of anti-MON antibody formation. When antibody titration was carried out with 2-ME treated sera, all these anti-POL responses were found to consist only of IgM response on day 5 and mostly of IgG response after day 15. Since the suppression by DMP was remarkable after day 15, we considered that DMP suppresses anti-POL IgG response mainly.

It is evident from another experiment in our laboratory (6) that anti-POL response in normal mice is induced through T cell dependent pathway in addition to T cell independent pathway and that anti-POL IgG response is T cell dependent. Therefore, we may conclude that DMP suppresses T cell dependent anti-POL response in the experimental system.

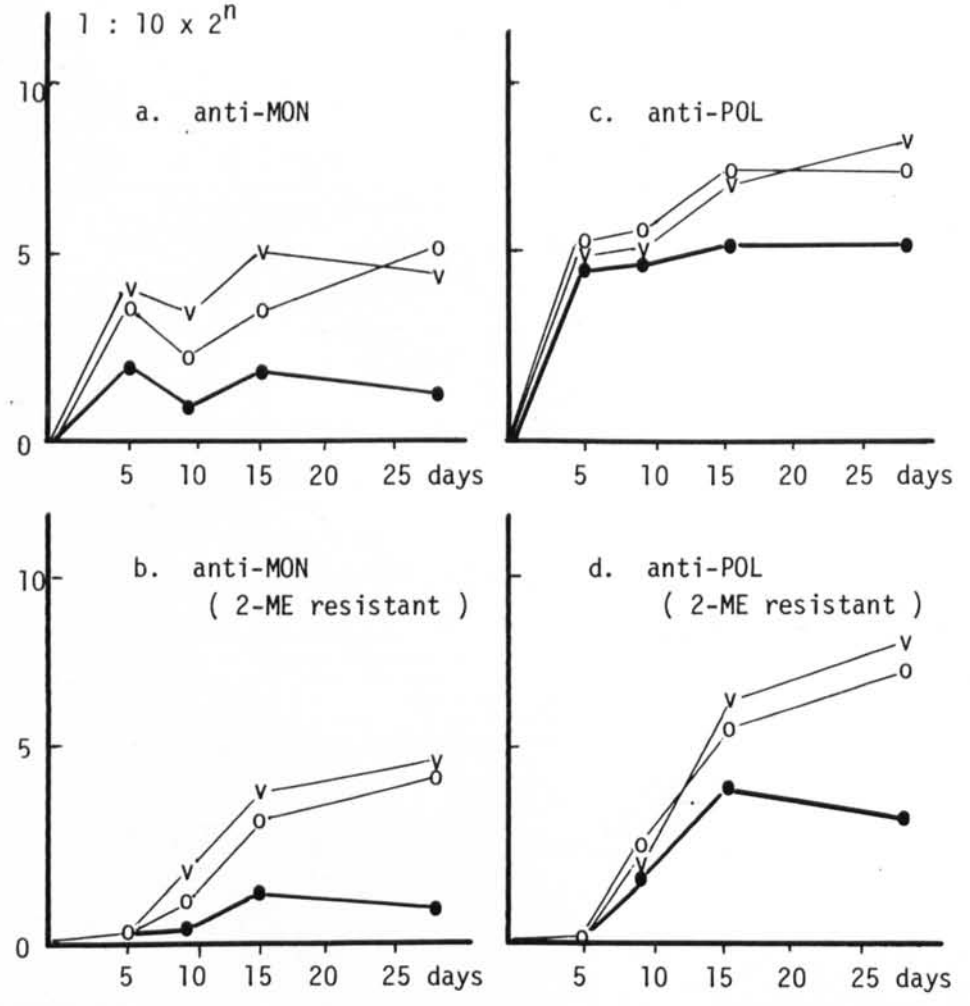

Fig. 1. The antibody response to MON (25 μg; a: 2-ME untreated, b: 2-ME treated) or to POL (10 μg; c: 2-ME untreated, d: 2-ME treated) in the mice receiving the following antigens : o——o : MON droplets or POL droplets ; v——v : MON+DR droplets or POL+DR droplets ; ●——● : MON+DMP droplets or POL+DMP droplets. Serum antibody titers are represented as the mean values of n in 1 : 10 x 2^n for each group of 5 mice.

Table I. IgM, IgG and IgE anti-DNP responses in the mice receiving DNP-KLH-DMP droplets.

Group of mice	Immunized with *	Serum antibody titer ** PHA on day 9 2-ME sensitive	2-ME resistant	PCA *** on day 15
1	DNP-KLH+DMP droplets + PBS droplets	7.8 ± 0.83	1.6 ± 0.54	1 : 10
2	DNP-KLH droplets + PBS droplets	8.0 ± 0.00	2.8 ± 0.50	1 : 40
3	DNP-KLH droplets + DMP droplets	8.8 ± 0.83	4.2 ± 0.83	1 :160
4	DNP-KLH solution	not detected		n. d.

* DNP_{120}-KLH : 10 μg per mouse. DMP : 1 mg per mouse.

** Serum antibody titer is represented as the mean of n values in $1 : 10 \times 2^n$ for each group of mice with standard error.

*** PCA was determined using rats according to Mota & Wong (7).

4. Suppressive effect of DMP on the IgE antibody response to DMP-KLH coexisting with DMP in the same oil droplets: As we have found that the immunization with an antigen in the form of w/o/w can induce IgE antibody response in mice, we carried out the following experiments in hopes to suppress an IgE antibody production specifically.

Four groups of five mice each were given DNP-KLH (10 μg/mouse) droplets plus PBS droplets, DNP-KLH+DMP (1 mg/mouse) droplets, DNP-KLH droplets plus DMP droplets and DNP-KLH solution, respectively. The anti-DNP antibodies detectable by PCA, 2-ME-sensitive and resistant, were produced almost equally in all mice except the group receiving free solution of DNP-KLH. The antibody detected by rat PCA, presumably of the IgE class (7), was significantly reduced in titer in the mice receiving DMP-KLH+DMP droplets, as shown in Table 1. The discrepancy between the suppression of IgM or IgG class antibody and that of IgE class is now under investigation, although it was shown by our experiments (2) that the production of an IgM or IgG class antibody against a hapten was hardly suppressed as far as DMP was used as an immunosuppressant.

DISCUSSION AND CONCLUSION

It was shown that the unresponsiveness to a given antigen can be induced by our method (2). For instance, the mice receiving BSA+DMP droplets plus HGG droplets were injected with BSA droplets plus HGG droplets plus SRBC (as a new antigen) on days 1, 5, and 7 after the priming, the antibody response to BSA was significantly reduced comparing with those of the control groups receiving BSA droplets plus HGG droplets or none, while the antibody to HGG or SRBC was produced in all the groups as expected.

Although we could not yet obtain the definite evidence to verify the hypothesis, it appears that one of the ways to achieve the selective immunosuppression to a given antigen was demonstrated. At this moment, we have not yet succeeded in the suppression of antibody being produced. It can be achieved by finding appropriate immunosuppressants, by using two or more drugs in combination or by clarifying the stage of sequence of cell-cell cooperation affected by various immunosuppressants. All these works are now under investigation.

ACKNOWLEDGMENT

This work was supported by the Science Research Grant of the Ministry of Education and of Health and Welfare and by the U.S.-Japan Cooperative Medical Science Program.

REFERENCES

1. Matuhasi, T. and Usui, M. Specific suppression of immune response to a given antigen by emulsified oil droplets containing a mixture of antigen and dexamethasone phosphate. Nature, New Biol. 245 (1973) 213.

2. Matuhasi, T., Usui, M., Minami, M., Kamei, J. and Hatakeyama, S. Studies on specific suppression of immune response to a given antigen I. Proc. Jap. Soc. Immunol. 2 (1973) 324.

3. Minami, M., Kanno, T., Kimura, J., Usui, M., Matuhasi, T. and Hatakeyama, S. Studies on specific suppression of immune response to a given antigen II. Proc. Jap. Soc. Immunol. 3 (1974) 267.

4. Miller, J. F. A. P. and Mitchell, G. F. Thymus and antigen-reactive cells. Transplant. Rev. 1 (1969) 3.

5. Moller, G. and Mitchell, G. F. Frequency of antigen sensitive cells to thymus-independent antigens. Cell. Immunol. 2 (1971) 309.

6. Minami, M., Kanno, T., Usui, M., Tamura, N., Watanabe, T. and Matuhasi, T. Requirement of T cells for the IgG and IgE antibody responses to thymus-independent antigens. Proc. Jap. Soc. Immunol. 4 (1975). (In press)

7. Mota, I. and Wong, D. Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. Life Science 8 (1969) 813.