Suppression of the secondary immune response by specific antibody, when given together with the secondary antigenic stimulus[1]

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Summary. It is generally believed that antibody-mediated immunosuppression can be only produced in non-primed individuals, and that this applies both to experimental animals and Rh-negative women at risk. However, in this paper it is reported that the additional injection of 0.2 ml of an antiserum to sheep erythrocytes (SE) together with a secondary antigenic stimulus of 10^8 SE into mice, primarily immunized by a tiny dose of 5×10^5 SE 28 days before, was capable of producing effective suppression of the secondary immune response.

The primary immune response is completely inhibited by administration of IgG antibody specific for the antigen[3–5], while the secondary is not[6–9]. It has been shown that priming for the secondary response is not inhibited at all, when the passive IgG antibody is given as early as 36 h after the primary injection of a 'saturated' dose of 10^8 sheep erythrocytes (SE) in mice[10]. On the other hand, priming for the secondary response was found to be still suppressed, when the IgG antibody was administered within a period of 72 h after the primary immunization with a very small dose of 5×10^5 SE[11]. Although such tiny doses of SE were found to be not capable of eliciting a measurable primary immune response at the cellular level, they will produce effective priming for the secondary response[11].

Advantage is taken of the phenomenon of antibody-mediated immunosuppression to prevent Rh sensitization of Rh-negative women at risk[12,13]. Analogously to the situation in experimental animals[11], it is generally believed that IgG anti-Rh has to be administered within a period of 72 h after delivery in nonprimed women[14,15]. It was, however, suggested that inhibition of sensitization is still achieved, when the anti-Rh is injected as late as 4–5 days after delivery[16,17]. But in spite of the suppression of anti-Rh formation demonstrated under such conditions in humans, it remains uncertain whether the preparation of the lymphoreticular tissues for the secondary response was prevented[18]. Besides the theoretical interest, it was therefore felt to be of some practical importance to find out whether or not antibody-mediated immunosuppression is still produced in mice, when the specific antibody is administered together with a secondary antigenic stimulus of 10^8 SE in mice primed several weeks before with a tiny dose of 5×10^5 SE.

Materials and methods. Mice. Adult female NMRI mice weighing 23–26 g were used. These specifically pathogen-free mice were obtained from the Central Institute for Laboratory Animals in Hannover (BRD).

Antigen. Doses of either 5×10^5 SE or 5×10^7 SE were used as a primary antigenic stimulus, whereas a constant dose of 10^8 SE was used for secondary immunization. Each dose was suspended in 0.2 ml of phosphate-buffered saline (PBS), pH 7.2, and administered by the i.p. route.

Specific allogeneic antiserum. Antisera to SE were obtained 7 days after NMRI mice had received the last of 4 i.p. injections of 4×10^8 SE at 14-day-intervals. In order to increase the process of priming, the first antigenic stimulation was performed by the simultaneous i.p. injection of 3×10^9 killed Bordetella pertussis cells, as described elsewhere[19]. The individual anti-SE sera were pooled and stored at −20 °C. The haemagglutination titer of the pooled antiserum (reciprocal) was 10,240 and the hemolytic activity amounted to 1580 50% hemolysis units per ml of serum. As shown by further investigations[9] (sucrose gradient, 10–40%, 4 °C, 210,000 × g, 18h) almost the total amount belonged to the 7S fraction. A constant volume of 0.2 ml of anti-SE serum in the dilution of 1:2 was given i.p. 1 h before the primary or secondary antigenic stimulus.

Antibody plaque techniques. For the quantitative determination of plaque-forming spleen cells (PFC), the direct[20] and indirect[21] antibody plaque techniques were used, as described[22]. It is generally accepted that the direct PFC (DPFC) represent 19S producers, whereas the majority of indirect PFC (IPFC) are considered to represent 7S hemolysin-producing cells[21,23].

Experimental procedures. Mice were primarily immunized with either 5×10^7 SE (group I) or 5×10^5 SE (group II). At the 28th day after primary immunization, the animals of both groups were divided into 2 subgroups, respectively. The mice of the subgroups I-A and II-A received at this time a secondary antigenic stimulus of 10^8 SE, whilst mice of the subgroups I-B and II-B were given

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2 Institute of Hygiene and Microbiology, University of Würzburg, BRD.

3 J. W. Uhr and G. Möller, Adv. Immun. *8*, 81 (1968).
4 H. Wigzell, Cold Spring Harb. Symp. quant. Biol. *32*, 507 (1967).
5 H. Wigzell, Antibiot. Chemother. *15*, 82 (1969).
6 D. A. Rowley and F. W. Fitch, in: Regulation of Antibody Response, p. 127. Ed. B. Cinader. Ch. C. Thomas, Springfield, Ill. 1968.
7 A. Morris and G. Möller, J. Immun. *101*, 439 (1968).
8 J. Sterzl, D. Johanovska and J. Milerova, Folia microbiol., Praha *14*, 351 (1969).
9 P. Emmerling, H. Finger and L. Eisele, Z. med. Mikrobiol. Immun. 156, 70 (1970).
10 H. Finger, P. Emmerling and I. Fölmer, Z. ImmunForsch. *142*, 468 (1972).
11 H. Finger and P. Emmerling, Z. ImmunForsch. *145*, 95 (1973).
12 V. J. Freda, J. G. Gorman and W. Pollack, Science *151*, 828 (1966).
13 WHO-Report, Bull. Wld Hlth Org. *36*, 467 (1967).
14 H. Finger and P. Emmerling, Immun. Infekt. *3*, 3 (1975).
15 P. Hindemann, M. Hinselmann and P. Frey, Gynaecologia, Basel *167*, 275 (1969).
16 G. Schellong and J. Schneider, in: Blutgruppenkunde, p. 111. Ed. B. Urbaschek. Die medizinische Verlagsgesellschaft, Marburg 1973.
17 J. Schneider, Dt. Med. Wschr. *101*, 596 (1976).
18 D. Samson and P. L. Mollison, Immunology *28*, 349 (1975).
19 H. Finger, P. Emmerling and E. Brüss, Can. J. Microbiol. *15*, 816 (1969).
20 N. K. Jerne, A. Nordin and C. Henry, in: Cell-Bound Antibodies, p. 109. Ed. B. Amos and H. Koprowski. Wistar Institute Press, Philadelphia 1963.
21 H. H. Wortis, R. B. Taylor and D. W. Dresser, Immunology *11*, 603 (1966).
22 H. Finger, P. Emmerling and E. Brüss, Infect. Immun. *1*, 251 (1970).
23 P. H. Plotz, N. Talal and R. Asofsky, J. Immun. *100*, 744 (1968).

0.2 ml of the anti-SE serum simultaneously with the booster dose of 10^8 SE. At different intervals after primary and secondary immunization, 6 mice out of each group and 2 untreated control animals were sacrificed and the spleens were removed.

Results. As can be seen form the data summarized in figure 1, the anti-SE serum, given together with the secondary antigenic stimulus of 10^8 SE in mice primed with 5×10^7 SE 28 days before, was not capable of influencing the secondary immune response. The peak values of IPFC determined at the 4th day following secondary immunization in the mouse group I-B (195,567 ± 36,484) did not differ significantly from those of the group I-A (285,083 ± 51,218). The same applies to the values of both direct and indirect PFC counted at all other examinations after secondary antigenic stimulation (figure 1). Figure 2 shows that the primary immunization of mice with 5×10^5 SE did not result in the development of PFC exceeding the numbers of pre-existing 'background' PFC. Nevertheless, this tiny dose caused effective priming, this being mainly documented by the finding that the secondary splenic response of the mouse group II-A was found to be characterized by the development of IPFC which had always prevailed, whereby the peak value (220,933 ± 46,713) became already detectable as early as 4 days after secondary antigenic stimulation. As compared to this, the peak value of DPFC (66,647 ± 13,570) amounted only to 31.7% of the corresponding peak number of IPFC (figure 2). From the data presented in figure 2, it is furthermore evident that the additional injection of 0.2 ml of anti-SE serum together with the secondary antigenic stimulus of 10^8 SE (group II-B) effected significant suppression of the secondary immune response. The numbers of both DPFC and IPFC determined in the spleens of the mice of group II-B were significantly lowered on all days tested (2 P at least ≤ 0.01) (figure 2). For example, the peak number of IPFC, as determined in the spleens of the mouse group II-B at the 4th day after boostering (6584 ± 2312) amounted to about 3% of the corresponding peak number of the mouse group II-A (220,933 ± 46,713).

Discussion. It is generally believed that antibody-mediated immunosuppression is effected only in non-primed individuals. This applies to experimental animals[6–9] as well as to Rh-negative women at risk[14,15]. In most experiments on the suppression of primary immune response by passively administered specific antibodies, optimal doses of antigens were employed. When passive antibodies were injected as early as 36 h after primary immunization of mice with 10^8 SE, the build-up of 19S and 7S immunological memory was not found to be impaired at all, indicating that competent precursor cells triggered for 36 h will react in a predetermined manner in a predetermined time without necessitating further antigen contact[10,14]. Thus, it cannot be surprising that the additional injection of 0.2 ml of anti-SE serum, together with a secondary antigenic stimulus of 10^8 SE into mice primarily immunized 28 days before with a suboptimal dose of 5×10^7 SE, did not influence the secondary immune response (figure 1), although such a dose was found to be capable of suppressing completely the immune response to a primary antigenic stimulus of 10^8 SE[9,14]. From this it becomes evident that committed progenitor cells representing immunological memory are much less susceptible – if at all – for the mechanism of antibody-mediated immunosuppression than the uncommitted precursors of antibody-producing cells. This is assumed to be due to the different avidity of antibody-analogous receptors on the surface of either committed or uncommitted competent precursor cells. With respect to that it is suggested that the receptors of committed cells possess

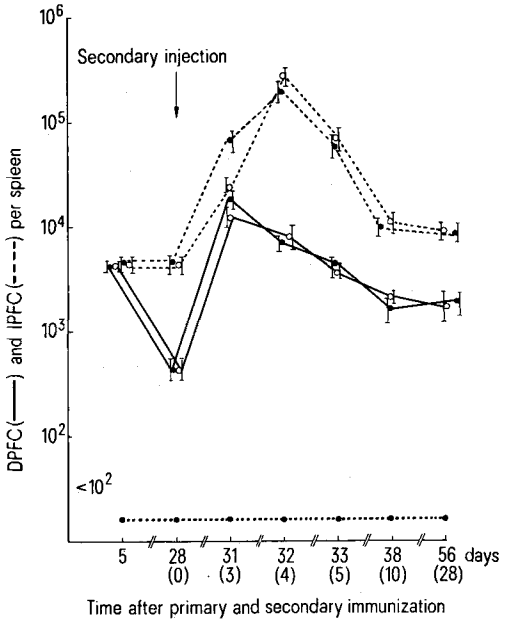

Fig. 1. Influence of specific antibodies on the development of direct (DPFC) and indirect (IPFC) plaque-forming spleen cells during the secondary immune response of mice to sheep erythrocytes (SE), when administered simultaneously with the secondary antigenic stimulus. Schedule of treatment: Mice of the group I were primarily immunized by the i.p. injection of 5×10^7 SE. Secondary antigenic stimulation by the i.p. route was performed 28 days later with either 10^8 SE (group I-A) (○) or 10^8 SE and 0.2 ml of anti-SE serum (group I-B) (●). 6 mice were used per point. . . . ., Untreated control.

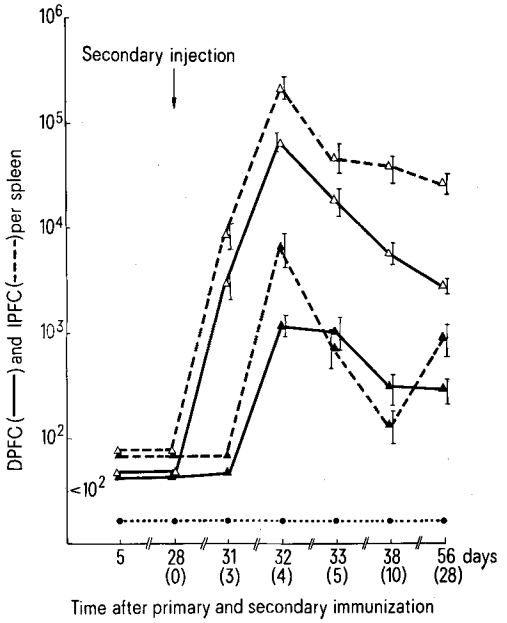

Fig. 2. Influence of specific antibodies on the development of direct (DPFC) and indirect (IPFC) plaque-forming spleen cells during the secondary immune response of mice to sheep erythrocytes (SE), when administered simultaneously with the secondary antigenic stimulus. Schedule of treatment: Mice of the group II were primarily immunized by the i.p. injection of 5×10^5 SE. Secondary antigenic stimulation by the i.p. route was performed 28 days later with either 10^8 SE (group II-A (△) or 10^8 SE and 0.2 ml of anti-SE serum (group II-B) (▲). 6 mice were used per point. . . . ., Untreated control.

stronger avidity than the uncommitted cells[24,25]. Assuming that the differentiation of uncommitted precursor cells, as well as the occurrence of antibody-producing cells, distinctly depends on the dose used for primary antigenic stimulation[26], and making the additional assumption that the passively administered 7S antibody is capable of inhibiting the build-up of immunological memory up to a certain degree of the differentiative processes of uncommitted progenitor cells, one may suggest that priming with tiny doses of antigens, such as 5×10^5 SE, leads to the formation of memory cells still carrying antibody-analogous receptors with relatively low avidity. Under those prerequisites one might expect that the specific 7S antibody passively administered together with a booster dose of 10^8 SE is capable of producing effective suppression of the secondary immune response. The findings obtained (figure 2) support this concept. Such experiments with tiny doses of antigens as a primary antigen stimulus are evidently very similar to the natural conditions of Rh-negative women at risk. The question arising which volume of fetal blood with Rh-positive erythrocytes will effect sensitization of Rh-negative women has not been answered by different authors in the same way[27]. This is not surprising, since it has been learned more recently that the immunogenicity of the D antigen of human erythrocytes from different donors will show considerable variance in human volunteers[28,29]. Taking into consideration that on one hand the individual sensitization risk of a Rh-negative nullipara by her Rh-positive child depends on the number of fetal erythrocytes present in maternal circulation immediately after delivery[30], and that on the other hand there exists no method for an exact determination of the volume of transfused foetal blood[13,31], it appears, on the basis of the experimental findings reported here, to be still worthwhile to administer the anti-Rh even if delivery has occurred more than 72 h previously.

24 G. Möller, Immunology *17*, 227 (1969).
25 G. W. Siskind and B. Benacerraf, Adv. Immun. *10*, 1 (1969).
26 A. M. C. Koros and E. C. Hamill, Immunology *25*, 717 (1973).
27 H. Finger and P. Emmerling, Dt. Med. Wschr. *95*, 1025 (1970).
28 H. H. Gunson, F. Stratton and P. K. Phillips, Br. J. Haemat. *32*, 317 (1976).
29 H. H. Gunson, F. Stratton and P. K. Phillips, Br. J. Haemat. *32*, 331 (1976).
30 P. L. Mollison, Br. J. Haemat. *14*, 1 (1968).
31 J. Schneider and O. Preisler, Blut *12*, 4 (1966).

Experimental allergic encephalomyelitis (EAE)-changes in structure and proliferation in rat lymph nodes after sensitization with guinea-pig and bovine basic myelin protein

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Summary. Substantial difference in the proliferation of lymphoid cells in the draining LN was found in rats injected with guinea-pig EBP-FCA and bovine NBP-FCA indicating significance of the encephalitogenic determinant in the myelin basic protein in the peripheral lymphatic reaction initiating EAE.

Regional lymph nodes (LN) draining the site of injection of the encephalitogenic antigen [basic myelin protein (EBP) mixed with complete Freund's adjuvant (FCA)] play a key role in the development of EAE. If such nodes are removed within 5 days after sensitization, no EAE develops[1]. It has been shown that the dynamics of growth of LN, as well as their histological structure, after administration of an EBP-FCA mixture differ markedly from changes produced by FCA alone[3]. The present work involves an attempt to specify the antigenic action of EBP on peripheral lymphatic reactions. To this end we have compared changes in structure and proliferation kinetics of regional LN after administration of encephalitogenic guinea-pig basic protein (EBP-FCA) and bovine basic protein (NBP-FCA) which does not induce EAE in the rat.

Cell proliferation in regional lymph node after injection of EBP-FCA, NBP-FCA and FCA alone, as compared with controls injected with saline

	EBP-FCA	NBP-FCA	FCA	Controls
Experiment I	11.3 ± 1.27*	5.5 ± 0.81	–	1.2 ± 0.10
Experiment II	10.9 ± 0.20	6.6 ± 1.05	5.8 ± 0.69	1.5 ± 0.01

*LI (%) ± SEM.

Material and methods. Female rats of the inbred Lewis strain, aged 10 weeks, were used. The EBP was prepared from guinea-pig brain by the method of Eylar[2]. The NBP was prepared from bovine material by the same method. Both antigens were injected intradermally into the left hind footpad in a dose of 400 µg per animal, dissolved in 0.1 ml saline and emulsified in the same volume of FCA (olive oil : lanolin : Tween 80, 15 : 4 : 1 and heat-killed Mycobacterium tuberculosis, 8 mg/ml adjuvans). Some animals received only FCA. Controls were injected with 0.2 ml of saline. On the 4th day after sensitization, the animals received i.p. 1 µCi 6-^{3}H thymidine/g b.wt (ÚVVVR, Prague, spec. act. 19–20 Ci/mMol); 60 min after injection, the lymph nodes were removed and processed as described elsewhere[3]. 2 and 7 µm thick slices were cut from the Paraplast-embedded material; 7 µm thick slices were stained with Mayer's hematoxylin and eosin. Autoradiograms were prepared from 2 µm thick slices as described elsewhere[3]. From each animal 2000–3000 cells were counted to determine the labelling index (L.I.).

1 R. M. Condie, in: Biology of Myelin, p. 321. Ed. R. S. Korey. Harper/Hoeber, New York 1959.
2 E. H. Eylar, H. Salk, G. Beweridge and L. Brown, Archs Biochem. Biophys. *132*, 34 (1969).
3 I. Matouš-Malbohan, M. Holub, J. Krásenská, V. Mareš and Z. Lodin, Cell. Immun. *12*, 350 (1974).