Overcoming of Endotoxin - Mediated Immunosuppression by *Bordetella pertussis* *

Endotoxins from gram-negative bacteria may act as adjuvants in the primary immune response[1,2]. This effect is evidently due to the multiplication of antibody-producing cells[3-6]. Furthermore, endotoxins significantly enhance the process of priming for the secondary response[7]. In contrast, it has been reported that the injection of relatively large amounts of bacterial lipopolysaccharides before the primary immunization with either antigen or antigen and endotoxin resulted in suppression of antibody formation instead of an enhanced response[8-11]. For the induction of this type of immunosuppression it appeared to be immaterial whether homologous or heterologous lipopolysaccharides were used for pretreatment and immunization[11]. The suppressive effect might be due either to an injury of the afferent limb of the immune apparatus or to reduction in progenitor immunocytes[11]. As compared with the adjuvancy of bacterial endotoxins, that of killed *Bordetella pertussis* cells was found to be considerably more pronounced[12,13]. Therefore it was suggested that adjuvancy of *B. pertussis* is only in part due to the action of the endotoxic structure[6]. If it holds true that structural components other than endotoxic lipopolysaccharides also produce adjuvant activity, it may be expected that those materials are capable of overcoming endotoxin-mediated immunosuppression. The experiments reported were carried out to test this effect.

Adult female mice of the strain NMRI (25–30 g) were used and divided into 3 groups. The animals of group I received an i.p. injection of 4×10^8 sheep red blood cells (SRBC) on day 0. Mice of both the other groups were additionally treated by the simultaneous i.p. injection of either 100 μg endotoxin (ET) from *Salmonella typhi* (Difco, *S. typhosa* 0901, Code 3124) (Group II) or 3×10^9 pertussis organisms (PO) (phase I, not adsorbed but killed with 1:10,000 dilution of Merthiolate for 30 min at 56 °C) obtained from the Behring-Werke Marburg, Germany (group III). Furthermore, mice of the groups II and III were pretreated with several i.p. injections of ET from *S. typhi*. Designating the day of immunization as day 0, 50 μg of the ET were given on days –5 and –4, 75 μg on day –3 and –2, and 100 μg on day –1, respectively. Mice of all groups were boosted with 4×10^8 SRBC 60 days after the primary immunization.

At different intervals after the primary and secondary immunization, 6–9 mice out of each group and 2 animals of the untreated controls were sacrificed, the spleens removed, the sera collected and after pooling stored at –20 °C until use. The numbers of 19 S and 7 S hemolysin-producing spleen cells were determined per 10^6 spleen cells and per total spleen, using the direct[14] and indirect[15] antibody plaque techniques as described elsewhere[12]. Total serum hemolysin activity of pooled serum samples and of fractions resistant to treatment with 0.125 *M* 2-mercaptoethanol (2-ME) were determined spectrophotometrically at 530 nm by the 50% hemolysis method.

As compared with the primary immunization of mice with 4×10^8 SRBC (group I), the additional treatment with increasing doses of ET from day –5 to day 0 (total amount: 450 μg ET) (group II) led to a significant inhibition of the primary immune response at the cellular and humoral levels, especially pronounced with respect to the indirect PFC (Figure). This is in accordance with previous findings[11]. In the group I the mean peak value of indirect PFC was found on day 10 amounting to 689,600 ± 69,500 per total spleen (2347 ± 295 per 10^6

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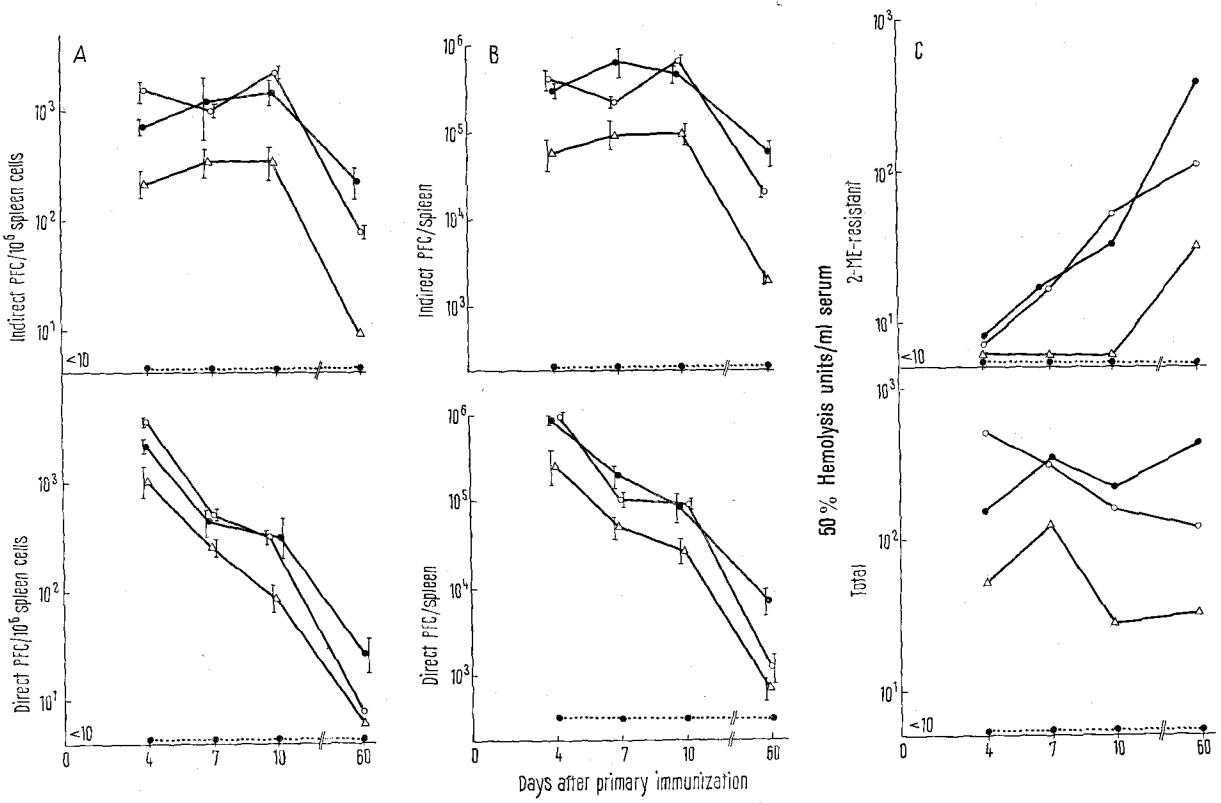
Development of direct and indirect plaque-forming spleen cells (PFC) during the secondary response of the mouse groups I–III [a]

Days after boosting	Average numbers of plaque-forming cells/10^6 spleen cells [b]					
	Direct PFC in the groups			Indirect PCF in the group		
	I	II	III	I	II	III
0 [c]	5± 2	3± 1	27± 10	79± 12	8± 1	230± 79
3	451± 84	157± 39	664±198	4195± 757	665± 199	4115± 605
4	745±189	576±143	1394±295	12139±1086	4343±1070	12196±2383
5	80± 11	319±112	1147±314	3922± 557	4936± 978	16282±3070
6	101± 24	130± 20	847±217	2470± 447	3049± 781	9042± 151

[a] The immunization schedule is given in the legend to the Figure. [b] The numbers represent the mean values and standard errors of 6 to 9 spleens. [c] Mice were boosted 60 days after the primary immunization.



Development of direct and indirect plaque-forming cells (PFC) per 10^6 spleen cells (A) and per total spleen (B) and total and 2-mercaptoethanol (2-ME) –resistant hemolysins (C) after the primary immunization of mice with 4×10^8 sheep red blood cells (SRBC) (○, group I), 4×10^8 SRBC and 100 µg endotoxin (ET) from *S. typhi* (△, group II) or 4×10^8 SRBC and 3×10^9 pertussis organisms (●, group III). The animals of the groups II and III were pretreated with relatively large amounts of ET from *S. typhi*. ....., untreated controls. 6 mice were used per point.

spleen cells). This differed significantly from the corresponding peak value of the group II (99000 ± 28000 per total spleen; 359 ± 116 per 10^6 spleen cells When 3×10^9 PO were injected simultaneously with the SRBC on day 0 into mice pretreated with ET from day −5 to day −1 (group III), the numbers of direct and indirect PFC were found to be significantly increased on all days tested in comparison to those of group II (Figure). The same applied to the serum hemolysin titers (Figure).

When mice of groups I to III received a secondary antigenic stimulus of 4×10^8 SRBC 60 days after the primary immunization, the secondary immune reaction was characterized by the predominant development of indirect PFC. Again, the spleens of the mouse group II contained significantly reduced peak numbers of indirect PFC, as compared with those of the group I (Table). This indicates that priming for the secondary response was considerably impaired in the animals of group II. On the contrary, the process of priming was found to be not impaired at all in the mouse group III, as can be seen from the data presented in the Table. Similar findings were obtained at the humoral level.

The results give evidence that pretreatment of mice with relatively large amounts of ET inhibits both the primary immune reaction and the process of priming for the secondary response. This indicates that besides the damage of immunocytes a reduction of antigen-processing may play a significant role in endotoxin-mediated immunosuppression. Since the latter could be completely overcome by the administration of *B. pertussis* cells, one may conclude that the capacity of these bacteria to function as nonspecific proliferative stimuli[6,12] is not solely due to their endotoxic structure, but, in addition, to other structural components. This concept is in accordance with the finding that the adjuvant activity of the whole organisms of *B. pertussis* was considerably greater than could be expected from its content of endotoxin[13].

Zusammenfassung. Die endotoxin-induzierte Suppression der Antikörperbildung gegenüber Schaferythrocyten beschränkt sich nicht nur auf die Primärreaktion, sondern findet ihren Ausdruck auch in einer signifikanten Verminderung der Präparation des lymphoretikulären Gewebes für die anamnestische Reaktion. Beide Suppressionseffekte lassen sich überwinden, wenn man den mit relativ hohen Endotoxindosen vorbehandelten Mäusen simultan mit dem Erythrocytenantigen abgetötete Zellen von *Bordetella pertussis* verabfolgt. Diese Aktivität dürfte darauf zurückzuführen sein, dass neben dem Endotoxin noch andere Strukturbausteine von *B. pertussis* adjuvante Aktivität besitzen.

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