INFECTION AND IMMUNITY, June 1973, p. 1009-1011
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Vol. 7, No. 6
Printed in U.S.A.

Enhanced Immune Response After Immunosuppression by Streptococcal Pyrogenic Exotoxin

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Received for publication 8 January 1973

Streptococcal pyrogenic exotoxin (SPE) was shown previously to be a potent immunosuppressant. This paper presents data documenting that immunosuppression frequently was followed by elevated antibody and plaque-forming cell levels as noted in our earlier report. The data are interpreted as a differential effect of SPE upon suppressor T cells and secreting B cells.

A small amount of streptococcal pyrogenic exotoxin (SPE) (7.0 μg/kg) was shown to be highly immunosuppressive in American-Dutch rabbits (1.0 to 1.5 kg average weight) injected on three successive days, beginning 3 h after a single injection of sheep erythrocyte (SE) antigen (4). Suppression of anti-SE antibody biosynthesis was manifested by inhibition of both plaque-forming cells (PFC) and the corresponding serum hemolytic antibody ($C'H_{50}$ quantitative hemolytic units). Suppression endured for a few days after the final toxin injection, and, paradoxically, there were often greatly enhanced serum antibody concentrations in animals receiving the same treatment when tested at 10 to 12 days. So-called "rebound" effects, which were similar to our observations, had been noted after X-ray immunosuppression (3, 8) and were not understood. We were convinced, however, that the toxin-induced effect was biphasic, and suggested that the toxin could be affecting the dynamics of cellular differentiation and proliferation (Edgar E. Hanna, Ph.D. thesis, Univ. of Minnesota, Minneapolis, 1967). We now present data which appear to fit a recently described suppressor T cell concept (1, 6, 9), which involves a thymus-derived cell which cooperates or interacts with other cells to antagonize B cell differentiation, synthesis, and/or secretion. Alternatively, suppressor cells may interact directly with B cells to shut off synthesis. The suppressor T cell is presumably a functionally distinct cell from the helper T cell (2), which apparently synergizes with the B cell.

All preparations, materials, and methods were given previously in great detail (4). PFC secreting 7S antibody were estimated as described (7) by using a hyperimmune, SE-absorbed, goat antirabbit 7S immunoglobulin at a 1:50 dilution.

Table 1 shows clearly that SPE treatment results in a prolonged, sustained enhancement of the 19S antibody response, with respect both to B cell secretion (direct PFC) and to circulating antibody serum levels ($C'H_{50}$ units). This effect can be appraised more clearly in Fig. 1, which demonstrates the dynamics both of the early profound suppression by toxin at four days (4) and of reversal to a prominent enhancement at 10 days, which persists through 30 days. Note that 7 and 70 μg, which are four-day suppressive doses, are the stronger enhancing doses at 10 days and that the higher dose response (70 μg) reaches a peak at 20 days and persists at a distinctly high level at 30 days, whereas control levels drop essentially to that seen at four days.

Table 2 shows that the toxin-treated group at ten days has an average 74-fold greater number of antiglobulin-facilitated (7S) plaques than does the control group injected with antigen only. Sustained high serum antibody levels are also apparent in this separate experiment and are more obvious in Fig. 1.

We envision, as a current working hypothesis, that SPE, which was shown to be cytotoxic for spleen cells (5), may kill or otherwise inactivate both B cells and suppressor cells, and that the rate of recovery by B cells (antibody secretors) is much faster, allowing exceptionally high levels of synthesis before suppressor cells recover (Fig. 1; 10 days, c). The mechanism could operate either because of relatively

TABLE 1. *Enhanced late antibody responses after SPE[a] immunosuppression to a single SE[b] injection.*

Rabbit	19S Response[c]		
	Injected with	19S PFC/10^6 spleen cells (direct method[d])	Serum antibody[d] ($C'H_{50}$ units/ml)
H798	SE	140	870
H799	SE	2	95
H800	SE	51	174
I24	SE	117	2,290
I25	SE	2	58
H673	SE + toxin	50	2,035
H674	SE + toxin	415	7,744
H675	SE + toxin	505	19,935
H796	SE + toxin	66	7,740
H797	SE + toxin	548	11,361

[a] Streptococcal pyrogenic exotoxin: 7.0 μg, iv on days 0, 1, and 2.

[b] Sheep erythrocytes: 2×10^9, iv on day 0.

[c] Assayed on day 11.

[d] By the direct method, the mean ± standard error was 62 ± 28 and 317 ± 107 with SE and SE plus toxin, respectively. By the serum antibody method, the mean ± standard error was 697 ± 425 and 9,763 ± 2,949 with SE and SE plus toxin, respectively.

TABLE 2. *Enhanced late antibody responses after SPE[a] immunosuppression to a single SE[b] injection*

Rabbit	7S response[c]		
	Injected with	7S PFC/10^6 spleen cells (indirect, antiglobulin method[d])	Serum antibody[e] ($C'H_{50}$ units/ml[e])
I67	SE	4	214
I68	SE	13	2,570
I69	SE	4	1,070
I70	SE	3	2,510
I71	SE	0	83
I51	SE + toxin	640	56,300
I52	SE + toxin	53	1,660
I53	SE + toxin	545	22,400
I55	SE + toxin	243	9,500

[a] Streptococcal pyrogenic exotoxin: 7.0 μg, iv on days 0, 1, and 2.

[b] Sheep erythrocytes: 2×10^9, on day 0.

[c] Assayed on day 11.

[d] By the indirect, antiglobulin method, the mean ± standard error was 5 ± 2 and 370 ± 234 with SE and SE plus toxin, respectively.

[e] By the serum antibody method, the mean ± standard error was 1,289 ± 538 and 22,465 ± 12,062 with SE and SE plus toxin, respectively. Measures predominantly 19S hemolytic antibody.

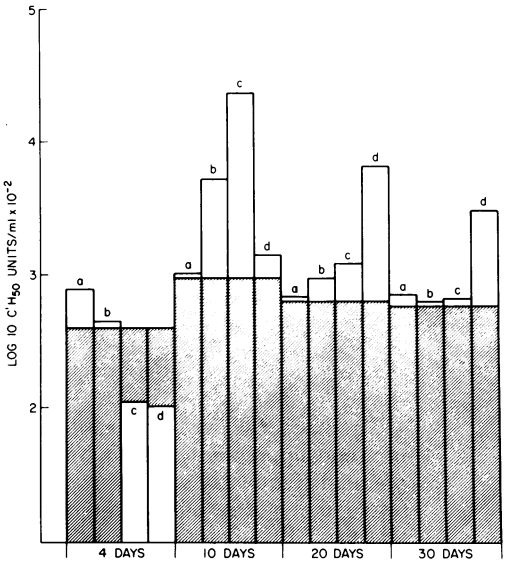

FIG. 1. *Dose-time response graph of streptococcal pyrogenic exotoxin (SPE) effects on anti-sheep erythrocyte (SE) antibody levels in rabbits injected once with 2 × 10^9 SE intravenously (iv). Test groups (open bars) were injected three times beginning 3 h after SE injections with SPE as follows: a, 0.07 μg iv; b, 0.7 μg iv; c, 7.0 μg iv; and d, 70 μg iv. Each bar on the graph represents the $C'H_{50}$ antibody titer of a serum pooled from at least four rabbits per group at 4, 10, 20, and 30 days after the SE injection. Cross-hatched areas represent the $C'H_{50}$ titer of a serum pooled as above from five control animals injected with SE and pyrogen-free saline only.*

greater susceptibility or a much smaller population of suppressor cells in the spleen or other lymphoid areas, or both.

This investigation was supported by Public Health Service grants HE-5360 from the National Heart Institute, currently the National Heart and Lung Institute, and AI-06487 from the National Institute of Allergy and Infectious Diseases.

We thank P. Leder and Y. B. Kim for criticizing the manuscript. We appreciate the expert secretarial preparations of C. Kunkle.

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