Short Communication

THE EFFECT OF BORDETELLA PERTUSSIS LYMPHOCYTOSIS-
PROMOTING FACTOR (LPF) ON ANTIBODY RESPONSE IN 
MICE: ITS ENHANCING AND SUPPRESSIVE EFFECTS

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SUMMARY: The effect of lymphocytosis-promoting factor (LPF) on antibody
response in mice was estimated under different sets of experimental conditions.
Four- and 6-week-old mice were intravenously inoculated with LPF. Three days
later these mice were inoculated either intraperitoneally or intravenously with sheep
red blood cell (SRBC) or human serum albumin (HSA) as an antigen.

The adjuvant effect of LPF was demonstrated on antibody response in 6-week old
mice to intraperitoneally inoculated SRBC but not to intravenously-inoculated one.
When 4-week-old mice were immunized, hemagglutinin production in response to
intraperitoneally inoculated SRBC was not enhanced by LPF. In addition, a rather
suppressive effect of LPF at a comparatively high dose was demonstrated on hemag-
glutinin production in response to intravenously inoculated SRBC.

Anti-HSA production was enhanced by inoculation of LPF in any combination
of the mouse age and the route of antigen administration.

These findings indicate that the adjuvant effect of LPF on antibody response in
mice depends upon experimental conditions: the age of mice, the quality of antigen
and the route of antigen administration used for immunization.

Several reports have shown that LPF of Bordetella pertussis acts as an
immunomodulator in humoral and cellular immunity. In these reports, however,
conflicting results were given in respect to the effect of LPF on antibody response
in mice. Asakawa (1969) demonstrated that antibody response of mice to SRBC
and tetanus toxoid was suppressed by inoculation of LPF prior to the antigen,
whereas some other workers observed an increased antibody response when LPF
was mixed with the antigen or given before the administration of antigen.
It is possible that the above contradictions may have been due to some different
experimental conditions used by them. To test this possibility, we compared
the effect of LPF on antibody response in mice under several different sets of
well-defined experimental conditions.

The LPF preparation used had been partially purified by the method de-
scribed previously (Iwasa et al., 1968). The preparation had high activities of LPF and histamine-sensitizing factor (HSF) as well; it contained little, if any, endotoxic activity. Either SRBC or HSA was used as an antigen. Conventionally-bred female mice (ddY-Shizuoka), 4 and 6 weeks old, were inoculated intravenously with LPF in a dose of 0.84 LPUₚ* of which lymphocytosis-promoting activity was comparable to those of doses used in our earlier work (Asakawa, 1969). Other mice given no LPF were served as control. Three days later these mice were immunized with an antigen intraperitoneally or intravenously and, thereafter, followed up for antibody response. Blood in an amount of 0.1 ml was taken from the left and the right retroorbital sinus alternatively at intervals of at least 7 days. The blood was diluted in 0.9 ml of saline for assaying anti-SRBC titer or in 0.4 ml for anti-HSA and allowed to coagulate overnight in a refrigerator. The retroorbital serums thus obtained were stored at -20°C until used for antibody titration. Hemagglutinin was titrated by the method described (Asakawa, 1969). Anti-HSA titer was determined by passive hemagglutination with glutaraldehyde-fixed mouse red cells sensitized with HSA by the aid of tannic acid (Bing, Weyand and Stavitsky, 1967; Kawaguchi, 1971). The reaction was allowed to proceed on microtiter plates with barbital buffer (pH 7.2) containing 1% heat-inactivated mouse serum as diluent. Retroorbital serums were inactivated at 56°C for 30 min prior to titration. Antibody titers were transformed into logarithm. A value of 5 was provisionally given to the titer of retroorbital serum showing negative anti-SRBC with undiluted serum, while a value of 2.5 was arbitrarily given to that showing negative anti-HSA with that. Significance tests were made at a probability level of p=0.05.

As the first step, the effect of LPF on anti-SRBC response was compared between 4- and 6-week-old mice (Fig. 1). A markedly increased hemagglutinin production was demonstrated in 6-week-old mice by inoculating with LPF when the antigen was injected intraperitoneally (Fig. 1a). Such a level of hemagglutinin production was also detectable in 6-week-old mice given LPF in a dose as low as 0.21 LPUₚ but not in a dose of 0.05 (data not shown). On the other hand, these 6-week-old mice responded to intravenously inoculated SRBC without any appreciable effect of LPF; no significant difference in hemagglutinin response was detectable between LPF-treated and -untreated mice (Fig. 1c). In 4-week-old mice, unlike 6-week-old ones, the administration of LPF failed to enhance hemagglutinin production in response to the intraperitoneally inoculated antigen (Fig. 1b). In addition, when SRBC was injected intravenously, the antibody

* 21 units of lymphocytosis-promoting activity (LPUₚ) have been assigned arbitrarily to a reference LPF preparation in this laboratory (Kurokawa et al., 1978). The activity of any preparation of LPF was determined relative to the reference by the parallel line assay method (Finney, 1964).

This activity unitage does not always correspond to that assigned to the national reference pertussis vaccine in use for "test for toxicity" for "precipitated purified pertussis vaccine" developed in Japan lately, because there was a significant deviation from parallelism between log dose-response regression lines of the reference LPF preparation and the national reference pertussis vaccine (Kurokawa et al., 1978).
Fig. 1. Effect of LPF on antibody response to intraperitoneally- or intravenously-inoculated SRBC.

Mice, 6 weeks (a, c) and 4 weeks (b, d) old, were immunized with SRBC in a dose of $5 \times 10^7$ cells 5 days after administration of LPF (○) or no LPF (△). Each point represents the mean of antibody titers of 5 mice and each vertical bar the confidence interval of the mean.

a, b: the antigen given intraperitoneally.
c, d: the antigen given intravenously.

response was rather suppressed by the inoculation of LPF (Fig. 1d); a decreased hemagglutinin production was observed as compared with that in mice given no LPF, in exact accordance with the previous findings obtained under similar experimental conditions (Asakawa, 1969). Such a suppressive effect of LPF was not detectable, however, in a dose as low as 0.21 LPU$_p$ (data not shown).

The next experiment was directed to the effect of LPF on antibody response to HSA instead of SRBC. A single shot of HSA in a dose of 0.1 mg produced a little, if any, antibody response by any route of antigen administration in
Fig. 2. Effect of LPF on antibody response to intraperitoneally- or intravenously-inoculated HSA.

Mice, 6 weeks (a, c) and 4 weeks (b, d) old, were immunized with HSA in a dose of 0.1 mg 3 days after administration of LPF (○) or no LPF (△). Each point represents the mean of antibody titers of 10 mice, except for those of 8 or 9 mice (see figures in parentheses) in some experiments, and each vertical bar the confidence interval of the mean.

a, b: the antigen given intraperitoneally.

c, d: the antigen given intravenously.

4-week-old as well as 6-week-old mice. LPF-treated 6-week-old mice, however, induced an increased antibody production regardless of the route of antigen administration (Fig. 2a, 2c). LPF-treated 4-week-old mice responded to the intraperitoneally inoculated antigen in a degree much the same as did the LPF-treated 6-week-old mice (Fig. 2b), while producing a detectable but fairly low anti-HSA response to the intravenously inoculated one (Fig. 2d). When LPF was reduced to 0.21 LPU_{p}, not only 6-week-old but also 4-week-old mice responded to HSA inoculated intraperitoneally or intravenously with an increased antibody production to the same extent as did 6-week-old mice given LPF in
a dose of 0.84 LPU<sub>p</sub> (data not shown).

These findings collectively indicate that the conflicting results reported on the effect of LPF on antibody response in mice should be interpreted from the different experimental conditions used by different workers. The workers who reported an adjuvant effect of LPF on antibody response did use mice of ages 8 to 10 weeks for immunization and adopted the ip route for antigen administration (Lehrer, Vangham and Tan, 1975a,b; Sato, 1976; Kamiyama, Sato and Sato, 1976), while Asakawa (1969) who observed a rather suppressive effect did use 4-week-old mice and adopted the iv route for antigen administration.

REFERENCES


