SUMMING UP, WEAK IMMUNOMODULATING SIGNALS OF TAHYNA VIRUS AND IMMUNOMODULATING DRUGS INDUCE IMMUNOSUPPRESSION IN MICE

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Summary. – The successive injection of non-immunomodulating doses of Tahyna virus (100 LD_{50}) and non-immunomodulating doses of immunomodulating drugs, such as purified staphylococcal toxoid or glucosaminylmuramyldipeptide (Likopid), to mice were accompanied by a decrease in the IgM plaque-forming cell response to sheep red blood cells.

Key words: Tahyna virus; immunosuppression; immunomodulators

To restore the impaired immune function in virus infection, many immunomodulating drugs have been proposed (Georgiev and Yamaguchi, 1993). Nevertheless, it is well known that viruses themselves are powerful immunomodulators inducing under certain conditions immunosuppression (Zschiesche *et al.*, 1987). For this reason we are faced with the question on the existence of an *in vivo* interaction between the immunomodulating signals of a virus and an immunomodulator. The data presented below give grounds to believe that such an interaction really exists. Our data demonstrate that the summation of weak immunomodulating signals of a virus and of an immunomodulator results in the development of immunosuppression.

Experiments were made on 6- to 8-week-old CBA mice obtained from the Stolbovaya Animal House (Moscow Region, Russia). Purified staphylococcal toxoid (PST) or glucosaminylmuramyldipeptide (Likopid) were used as immunomodulators (Semenova *et al.*, 1993; Ivanov *et al.*, 1996). Tahyna virus strain PV was obtained from the Chumakov Institute of Poliomyelitis and Viral Encephalitides.

The experimental protocol was as follows:

100 LD_{50} of Tahyna virus was inoculated into mice intraperitoneally (i.p.). Then an immunomodulator was injected in a single (on day 0 or 4 p.i.) or in several doses (see below). PST was inoculated i.p. in a dose of 0.015 or 0.15 binding units (BU) per mouse; Likopid was introduced orally in a dose of 0.1 μg or 1 μg. Four days p.i., the mice were injected i.p. with 2 x 10^7 sheep red blood cells (SRBC). The number of anti-SRBC IgM splenic plaque-forming cells (PFC) was estimated by the Jerne plaque assay (Jerne *et al.*, 1963) 4 days after the inoculation of SRBC. The data were expressed in number of PFC per 10^6 splenocytes. Each experiment was repeated at least twice. The statistical analysis was performed with the use of the Student's t-test.

As we can see from the data presented in Fig. 1, the PFC response of the animals injected with 100 LD_{50} of Tahyna virus, or 0.015 BU of PST or 0.1 mg of Likopid alone did not differ from that of the control animals. At the same time, the inoculation of 10-fold greater doses of immunomodulators (0.15 BU of PST or 1 μg of Likopid) induced a considerable increase in the number of PFC. The inocula-

Abbreviations: BU = binding unit; PFC = plaque-forming cells; i.p. = intraperitoneal(ly); p.i. = post infection; PST = purified staphylococcal toxoid; SRBC = sheep red blood cells

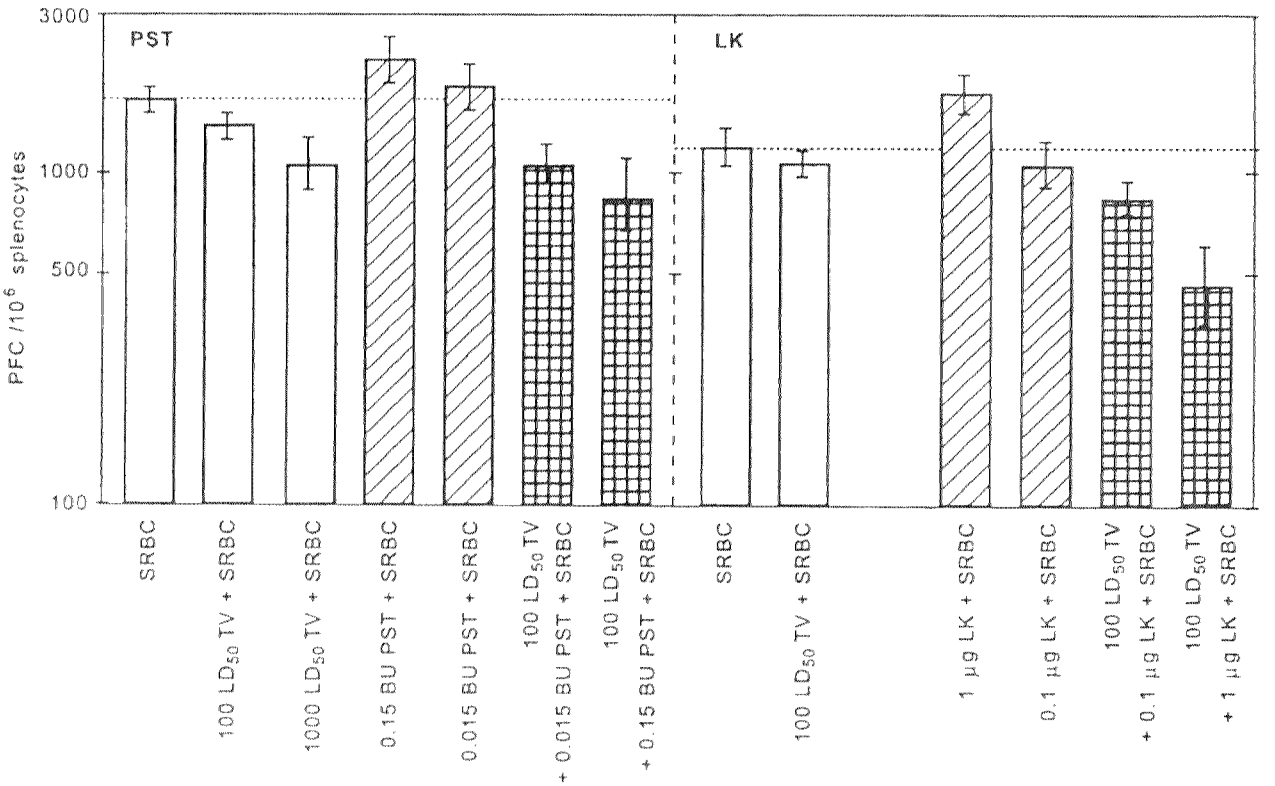

Fig. 1
Development of immunosuppression in mice treated with non-immunomodulating doses of Tahyna virus, PST or Likopid

tion of 100 LD_{50} of Tahyna virus and 0.015 BU of PST (or 0.1 μg of Likopid) four days later caused a marked decrease in the number of PFC as compared to the SRBC control. An immunosuppression also developed after the inoculation of a non-immunomodulating dose of Tahyna virus (100 LD_{50}), the adjuvant dose of immunomodulators (0.15 BU of PST or 1 μg of Likopid) four days later and SRBC four more days later. No significant change in the number of PFC was observed after a simultaneous injection of 100 LD_{50} of Tahyna virus and of 0.015 BU of PST or 0.1 μg of Likopid (data not shown).

In evaluating the data presented in this work the following facts investigation should be taken into consideration:

(1) 100 LD_{50} of Tahyna virus did induce a weak immunosuppression which could be detected after the inoculation of 2×10^6 instead of 2×10^7 SRBC. In this case, the infected mice developed 2 times less anti-SRBC PFC as compared with the non-infected control mice (227 and 577 PFC, respectively, $p \leq 0.05$; data not shown).

(2) The animals, infected with 100 LD_{50} of Tahyna virus subsequently treated with the doses of PST (0.015 BU) or Likopid (0.1 μg) not modulating immune response to 2×10^7 SRBC, demonstrated a decrease in their ability to develop anti-SRBC PFC as compared with the non-infected control mice.

(3) The animals infected with 100 LD_{50} of Tahyna virus showed a decrease in the number of anti-SRBC PFC after the inoculation of 0.15 BU of PST or 1 μg of Likopid. The virus was administered four days prior to the inoculation of the immunomodulators. As shown in our earlier experiments, these doses restored the immunosuppression induced by 1000 LD_{50} of Tahyna virus (Semenova *et al.*, 1992).

The data presented in this work suggest that a summation of the signals of different immunomodulators may occur *in vivo* under certain conditions. The summation of weak signals of the virus and the immunomodulator may lead to the development of immunosuppression. An immunosuppression also developed in cases of a combination of strong signals of an immunomodulator (restoring virus-induced immunosuppression) and weak immunomodulating signals of Tahyna virus.

It should be pointed out that the immunosuppression, associated with the weak immunomodulating signals of Tahyna virus and PST, did not develop if Tahyna virus (100 LD_{50})-infected mice were inoculated with 0.015 BU

of PST on days 0, 2, and 4 or on days 2, 4, and 6 p.i. The test and control animals were found to have 1499 and 1597 anti-SRBC PFC, respectively, in the first case, and 1327 and 1579 anti-SRBC PFC, respectively, in the second case ($p \geq 0.05$). The PFC response in mice, infected with 100 LD_{50} of Tahyna virus and treated with 0.1 μg of Likopid on days 2, 4, and 6 p.i. did not differ from that in the control animals (1807 and 2141 PFC, respectively, $p \geq 0.05$).

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