The Polybacterial Lysate Olimunostim Modulates Lymphocyte Function in Vitro and Restores Depressed Cellular Immunity in Vivo

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ABSTRACT. Immunobiological activity of the polybacterial lysate Olimunostim (P. acnes, K. pneumoniae, S. aureus) was examined by investigating its effects on murine lymphocytes. When added to in vitro lymphocyte cultures, Olimunostim induced interleukin-2 (IL-2) biological activity (in a 2-d culture) and subsequently potentiated lymphocyte proliferation (on day 3); the latter effect was dependent on the presence of adherent cells. In vivo, significant enhancement of lymphocyte reactivity to T-mitogens and increase of CD4+ helper-inducer T lymphocytes were observed 3 d after a subcutaneous application of Olimunostim to mice with cellular immune deficiency. These results confirm the modulatory properties of Olimunostim towards lymphocytes both in vitro and in vivo, which may form a basis for its clinical application.

Olimunostim is a polybacterial lysate composed of Propionibacterium acnes, Klebsiella pneumoniae and Staphylococcus aureus strains selected for their immunoenhancing activity (Weigl et al. 1989). It belongs to the group of bacterial preparations such as the polybacterial lysate Bronchovaxom, or the membrane glycoprotein extract of K. pneumoniae, Biostim. Despite the relatively vague chemical definition of these preparations (Vaněk et al. 1991), all of them - Bronchovaxom, Biostim and Olimunostim – have been successfully used in clinics mostly for treatment of recurrent respiratory infections (Palma Carlos and Palma Carlos 1990; Michel et al. 1980; Bystroň et al. 1992).

Recently, a series of experimental studies from this institution documented a wide range of immunobiological activities of Olimunostim in vivo including animal models of infection and malignancy (Weigl et al. 1992). For example, application of Olimunostim to healthy mice (Petfek et al. 1992) and also to mice infected by Salmonella typhimurium (Hajduš et al. 1992) resulted in potentiation of the (mostly cellular) immune response. In these situations, macrophages and lymphocytes were the main targets of immunopotentiating Olimunostim activity.

We characterized the effects of Olimunostim on key immune cells, lymphocytes, in vitro and also in an animal model of immune deficiency. In vitro, effects of Olimunostim on IL-2 production and on lymphocyte proliferation were investigated using cultures of murine splenic lymphocytes. In vivo, a murine model of selective T lymphocyte deficiency was employed to explore if Olimunostim could restore depressed lymphocyte reactivity to T-mitogens and compensate for decreased T lymphocyte numbers.

MATERIALS AND METHODS

Olimunostim

Lysates of the bacterial strains comprising Olimunostim (P. acnes strain 16073, S. aureus strain 1239 and K. pneumoniae strain 1129; strain numbers according to the Collection of Microorganisms, Brno, Czech Republic) were prepared as described by Weigl et al. (1989) and supplied as lyophilized substances (Biotechnology Division, Palacky University, Olomouc). The substances were dissolved in phosphate-buffered saline to yield stock solutions. To prepare Olimunostim, the stocks of P. acnes, S. aureus and K. pneumoniae were pooled in a 1:1:1 ratio.

The lyophilized substances of Olimunostim components were prepared as follows. Using small-volume (1.5–10 L) bioreactors (Sulzer, Switzerland), S. aureus and K. pneumoniae were cultivated in a culture medium based on the Todd-Hewitt broth (Imuna, Šarišské Michaňany, Slovakia); P. acnes was cultivated in VLBroth (Imuna, Šarišské Michaňany, Slovakia) under anaerobic conditions. Bacterial biomass was isolated using an MBR Dynamic Bio Pressure Filter (type BDF-01, Sulzer, Switzerland; pore size 0.2 µm). After washing the biomass in saline, the resulting sediment was suspended to a concentration of 40–80/pL in distilled water. S. aureus and K. pneumoniae strains were subjected to alkaline lysis (pH 9.6) until the loss of bacterial stainability; the average duration of the
lysing period was 3 d. All three bacterial components of Olimunostim were inactivated by repeated heating to 60 °C and then subjected to individual lyophilization.

**Experimental animals**

Female, 6–8-week old Balb/c mice (*Velaz, Lysolaje, Czech Republic*), were used. Healthy mice served as a source of splenic cells for studying Olimunostim properties in vitro; for the procedure of splenocyte isolation see Petřek *et al.* (1992). Other mice were subjected to testing of Olimunostim effects in vivo. The care of the animals and the experiments complied with the Czech law.

**In vitro experiments**

*Olimunostim effect on lymphocyte proliferation.* Lymphoproliferative effect of Olimunostim, and also of its components, was investigated (1) in crude suspensions of splenocytes with lymphocytes and macrophages, and (2) in purified suspensions of non-adherent cells, i.e. lymphocytes. Lymphocyte transformation test (*e.g.* Petřek *et al.* 1992) was used to obtain a quantitative assessment of Olimunostim-induced changes of lymphocyte proliferation. The cells (100 000 per well) were cultivated either with the medium alone (control) or with Olimunostim (dose range 0.03–50 μg/L) for 1, 2, 3, 4 and 5 d. Five hours before the end of the cultivation 3H-thymidine (18.5 kBq per well) was added and the proliferation was quantified according to the rate of 3H-thymidine incorporation expressed as counts per min (cpm). The cpm values were converted to stimulation indices (SI), which compared proliferation in Olimunostim-stimulated cultures to that in the control culture (SI = cpmOLIMUNOSTIM : cpmCONTROL).

*Olimunostim effect on IL-2 production.* IL-2 was identified in the supernatants of Olimunostim-stimulated and control cultures using a biological assay with the IL-2 dependent cell line CTLL-2 (Gillis *et al.* 1978); kindly provided by Dr. Robinson (*University of Newcastle upon Tyne, UK*). To illustrate the effect of Olimunostim on IL-2 production, the s.c. "Stimulation/Control IL-2 ratio" (R) was calculated: cpm values of CTLL-2 proliferation induced by supernatants from Olimunostim-stimulated cultures were divided by values of CTLL-2 proliferation induced by the control supernatant aspirated from non-stimulated culture. IL-2-inducing effects of Olimunostim components were tested in the same way.

**In vivo experiment**

*Effects of Olimunostim in an animal model of T lymphocyte deficiency.* Balb/c mice (*n* = 48) were administered two doses (1 mg/kg) of anti-Thy 1.2 monoclonal antibody to elicit a selective T cell immune deficiency (Nouza *et al.* 1990; Petřek 1992). The dose and the application schedule (2 d and 1 d prior to the determination of immunological parameters; Fig. 1), were determined in preliminary experiments (*data not shown*). In parallel with the second dose of the antibody, the mice received Olimunostim (20 mg/kg s.c.) and the following immunological parameters were determined: (1) mitogen-induced lymphocyte proliferation using a standard lymphocyte transformation test with phytohaemagglutinin (PHA) and concanavalin A (Con A), (2) subpopulations of splenic T lymphocytes by indirect immunofluorescence using rat monoclonal antibodies against murine CD4 and CD8 (antibodies were donated by Dr. Brooks, *University of Newcastle upon Tyne, UK*); for details of the methods see Petřek *et al.* (1992).

![Fig. 1. Scheme of an experiment evaluating Olimunostim activity in an animal model of a selective T-lymphocyte deficiency; S – application of the antibody against T lymphocytes, M – Olimunostim application, D – determination of the parameters under study. For further details see Materials and Methods.](image-url)

To assess the extent of Olimunostim-induced changes, the parameters in the group of Olimunostim-treated immunodeficient animals (*n* = 24) were compared with those in the controls, i.e. immunodeficient animals not exposed to Olimunostim (*n* = 24), and also with the parameters recorded in healthy animals (*n* = 12).