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Mechanism of T-cell Specific Immunosuppression Induced by Prodigiosin

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***ABSTRACT**: In a series of our screening for immunomodulating substances, we isolated prodigiosin from the culture broth of Serratia marcescens B-1231. This compound inhibited the T cell-mediated immune responses such as concanavalin A-induced proliferation, mixed lymphocyte response, local graft versus host reaction and T-dependent antibody response at nontoxic concentrations. However, prodigiosin did not affect B cell-mediated immune functions such as lipopolysaccharide-induced proliferation and -activated polyclonal antibody production at the same concentrations. Prodigiosin did not cause death in vitro to lymphocytes at effective concentrations (<100 nM) and also did not show toxicity in vivo to lymphoid organs at effective dosages (10 and 30 mg/kg). The pharmacological potencies were comparable to the activities of well-known T-cell specific immunosuppressants such as cyclosporin A. In our continuing study, mechanism of action of PDG is investigated with respect to the effect of PDG on IL-2/IL-2R pathway and transcription factor.*

1. Introduction

Recently, immunomodulators, especially suppressants, made a number of valuable contributions in the improvement of immunosuppressive therapy, in the context of organ and tissue transplantation and in our awareness of immunological phenomena which underlay and might be crucial for the acceptance of organ transplantations. Cyclosporin A, cyclophosphamide, rapamycin, and FK506 were included in this category and clinically studied or used in organ transplantation. However, the developments of new immunosuppressants, which have a novel spectrum of actions, are needed.

In the course of our screening of immunomodifiers, the red pigment, prodigiosin, was isolated from the culture broth of *Serratia marcescences* B-1231. The red pigments, produced by microoraganisms including *Streptomyces* and *Serratia*, are a kind of polypyrroles possessing a common, characteristic pyrrolylpyrromethene. They include many related compounds such as prodigiosin, prodigiosin 25-C, metacycloprodigiosin, prodigiosene, and desmethoxyprodigiosin. Prodigiosin and some of the related compounds showed potent antimicrobial, antimalarial and cytotoxic activity. One of the related compounds, prodigiosin 25-C has been extensively studied as an immunosuppressive agent and it has been found to be more suppressive of the functions of T cells than those of B cells. Although a related compound, prodigiosin 25-C, was studied for its immunomodulating and other biological activities, the immunomodulating activities of prodigiosin had not been studied. These facts prompted us to study the effects of prodigiosin on the immune system *in vitro* and *in vivo*. In this study, we evaluated the immunosuppressive activities of prodigiosin. The functions of T and B cells were determined separately to address whether prodigiosin showed T cell specific suppression like other immunosuppressants. Also we determined the *in vitro* and *in vivo* toxicity.

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2. Isolation of prodigiosin from culture broth of *Serratia marcescences*

During the course of the screening program to find immunomodulators from microbial sources, an active compound was isolated from the culture broth of bacterial strain B-1231, which was isolated from a

marine sample from Mokpo, Chunnam Province, Korea. It was taxonomically identified as *Serratina marcescences*. An active material was purified by ethyl acetate extraction and silica gel column chromatography. By spectroscopic analysis, the compound was proved to be prodigiosin (molecular weight, 323 and formular $C_{20}H_{25}N_3O$) and had a structure similar to prodigiosin 25-C.

3. Suppression of T and B cell proliferation by prodigiosin

Splenic lymphocytes were cultured with prodigiosin (0.3 to 30,000 nM) from Day 0 to Day 3. At below 100 nM, prodigiosin did not induce cell death. However, above 300 nM, the viability of lymphocytes rapidly decreased starting on Day 2.

Prodigiosin suppressed T cell proliferation induced by concanavalin A (Con A, 5 μg/ml) at concentrations higher than 3 nM and a phenomenal suppression was observed at 30 nM. The proliferation of lymphocytes induced by pokeweed mitogen (PWM, 5 μg/ml), known as a T and B cell common mitogen, was also suppressed starting at a concentration of 10 nM and was strongly suppressed at 100 nM. However, B cell proliferation induced by lipopolysaccharide (LPS, 5 μg/ml) was not affected by prodigiosin at concentrations up to 100 nM. These results suggest that prodigiosin selectively inhibited the blastogenesis of T cells but not that of B cells in noncytotoxic concentrations.

4. Suppression of the immune functions of T and B cells by prodigiosin *in vitro*

The antibody production of B cells activated by polyclonal B cell stimulant, LPS (25 μg/ml) which activated B cells to antibody producing cells, was evaluated on Day 3 after adding LPS and prodigiosin (0.3~100 nM). The antibody production of B cells was not changed by prodigiosin at the present concentration ranges. T-dependent antigen, sheep red blood cells, was chosen as another antigen to immunize B cells because this antigen needed other cells such as helper T cells and antigen-presenting cells to immunize B cells. The antibody production of B cells was suppressed by 3 nM prodigiosin and strongly suppressed at 100 nM prodigiosin. The primary T cell activation by two-way mixed lymphocyte response (MLR) in which only T cells responded to MHC molecules of other cells was determined. Prodigiosin suppressed T cell activation beginning at 30 nM and strongly suppressed it at 100 nM. As expected in the results of blastogenesis assay, prodigiosin strongly suppressed T cell functions but not B cell functions.

5. Subset analysis

Even though the viability of total lymphocytes was not influenced by prodigiosin at concentrations from 0.3 to 100 nM, the selective toxicity of T cells might occur in the context of the selective suppression of T cells. This possibility was examined by flow cytometric analysis. Prodigiosin was treated *in vitro* for 3 days. The percentages of B and T cell subsets in total cell population are not changed by prodigiosin at concentrations up to 100 nM, suggesting that the optional suppression of T cell functions does not result from selective toxicity of T cells.

6. Suppression of *in vivo* T-dependent antibody response by prodigiosin

T-dependent antibody response to SRBC which required the participation of T, B, and antigen presenting cells were also suppressed by the *in vivo* treatment of prodigiosin. Prodigiosin inhibited the production of antibody forming cells by 68.4% and 72.6% of control at 10 and 30 mg/kg, respectively. Prodigiosin seemed to be nontoxic which was probably supported by the ratio of spleen to body weight.

7. Suppression of graft versus host reaction (GvHR) by prodigiosin

GvHR was a T cell specific response and was induced by the immunorejection between allogeneic strains. Prodigiosin inhibited the enlargement of popliteal lymph nodes by 68.4% and 72.3% of control at 10 and 30 mg/kg, respectively. The positive control was cyclophosphamide (100 mk/kg), which strongly inhibited the GvHR. The treated animals did not show any toxicity and their body weight did not decrease.