40 Effects of *Pseudomonas aeruginosa* PA-I and PA-II Lectins on Tumoral Cells

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*Pseudomonas aeruginosa* is an opportunistic pathogenic Gram-negative bacterium. It is unique in its ability to withstand the antimicrobial activity of the currently used antibiotics and other drugs. As a result, this bacterium, which had been formerly used to cure streptococcal infections, has gained the status of a serious medical problem (Sabath 1980; Cryz 1984). It endangers the life of hospitalized patients suffering from extensive burns, chronic lung (e.g., cystic fibrosis), kidney and other diseases, as well as patients exhibiting congenital, acquired (e.g., AIDS), or therapeutically induced (e.g., cancer patients following chemotherapy or radiotherapy and subjects treated for organ transplantation) immuno-deficiency (Sabath 1980; Cryz 1984). The high virulence of this bacterium is due to two groups of products: (a) sophisticated virulence factors and (b) lectins plus lectinoid adhesins (Gilboa-Garber and Garber 1992). Protection against *P. aeruginosa* infections has therefore been sought in vaccines (Sabath 1980; Cryz 1984) that stimulate specific immunity against the bacterium toxic products (for prevention of their effects) or against its lectins, or other adhesions, which enable the first step of infection via the adhesion of the bacterium to the host cells.

*P. aeruginosa* produces high levels (comparable to those found in legume seed extracts) of two lectins, PA-I and PA-II, that resemble the phytohemagglutinins in their specificity, properties and applications (Gilboa-Garber 1988; Gilboa-Garber and Garber 1992). These two lectins, the first galactophilic and the second specific for l-fucose and d-mannose, were purified using affinity chromatography (Gilboa-Garber 1982). The purified lectins have been used for stimulation of human peripheral lymphocytes (Gilboa-Garber 1988; Gilboa-Garber and Garber 1992) and murine splenocytes (Avichezer and Gilboa-Garber 1987) and for protection of mice (as a very effective vaccine) from otherwise lethal *P. aeruginosa* infections (Gilboa-Garber 1988; Gilboa-Garber and Garber 1992). Recently, the gene coding for PA-I lectin was isolated and sequenced and the amino acid sequence of the PA-I lectin subunit was deduced (Avichezer et al. 1992).

The PA-I and PA-II lectins were found by us to be of medical importance for cancer diagnosis when applied for the estimation of peripheral T lymphocyte mitogenic response (instead of the classical mitogenic lectin of *Phaseolus vulgaris*) using thymidine incorporation assay (Gilboa-Garber et al. 1986). They have also been used in the field of experimental cancer (AKR lymphoma and Lewis lung carcinoma) cell suppression (Avichezer and Gilboa-Garber 1991). The interaction of the *P. aeruginosa* lectins with the cancer cells was shown by agglutination tests, by suppression of the tumor cell viability and growth in vitro, and by reduction of its tumorigenicity (but preservation of immunogenicity) in vivo (Gilboa-Garber et al. 1986, Leibovici et al. 1987, Avichezer and Gilboa-Garber 1991). The most interesting and important were the observations on the PA-I and PA-II lectin diverse, but complementary effects on Lewis lung carcinoma cells in vitro (Avichezer and Gilboa-Garber 1991); while PA-I had a direct suppressive effect, PA-II effect was mediated by activation of cytotoxic immune cells (e.g., lymphocytes, macrophages,
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polymorphonuclear and natural killer cells). Deduction from the results in mice to human beings is still premature; however, such a possibility exists, since recent findings indicate that both PA-I and PA-II lectins interact with different human blood group antigens, including those regarded as associated with cancer such as P, T and Tn, Le^a, and Le^b, and the stage-specific embryonic or oncogenic antigens (SSEA) of different types (Levene et al. 1993). An appropriate combination of PA-I and PA-II lectins may function synergistically as antitumor therapy, leading to tumor cell destruction by acellular and cellular immune mechanisms, and provide, at the same time, protection against *P. aeruginosa* infections which endanger cancer patients.

The present chapter describes the direct effects of PA-I and PA-II lectins on AKR lymphoma and 3LL cells exhibited in cell agglutination, reduced survival and proliferation in vitro, as well as decreased tumorigenicity in vivo (with preserved immunogenicity). It also describes the in vitro, cell-mediated antitumoral effect of PA-II lectin on 3LL cells.

### 40.1 Murine Tumoral and Normal Cells

The murine tumoral cells used are AKR lymphoma cells, which induce tumors in inbred AKR/Cu mice, and Lewis lung carcinoma (3LL) cells, which induce tumors in inbred C57B1/6J mice. The normal murine cells used are splenocytes (both as a control for the lymphoma cells and as responder immune cells for the cell-mediated antitumoral effect on the 3LL cells) and lung cells (as a matching for the 3LL cells).

#### 40.1.1 AKR Lymphoma Cells

The two variants of AKR lymphoma cells used, one exhibiting low malignancy (LM) and the other high malignancy (HM), were described by Gross (1957). The first produces large subcutaneous (s.c.) tumors and no (or small) metastases in the lymph nodes, and the latter is highly metastatic in AKR/Cu mice (Leibovici et al. 1987).

AKR lymphoma cell suspensions are prepared from subcutaneous (LM) and mesenteric lymphatic (HM) tumors produced in AKR/Cu mice. These tumors are removed aseptically, excised, and pressed through a sterile 50-mesh stainless steel screen (with the aid of a plunger from a 5-ml syringe) into 5 ml of Medium A (antibiotic-containing medium) composed of RPMI 1640 medium (Gibco) supplemented with 100 units penicillin G, 100 μg dihydrostreptomycin and 0.2 μg p-hydroxybenzoic acid butyl ester/ml. Cell clumps are removed by sedimentation. The unclumped cell suspensions are washed twice in Dulbecco's phosphate buffered saline (DPBS) [prepared as follows: (a) NaCl 8 g, KCl 0.2 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.2 g in 800 ml water; (b) CaCl_2 0.1 g in 100 ml water; (c) MgCl_2·6H_2O 0.1 g in 100 ml water. The (a), (b), and (c) solutions are autoclaved separately, cooled, and then mixed] by centrifugation (100g for 7 min in a clinical centrifuge) and resuspended in RPMI medium (when used for both routine cell maintenance and in vivo experimental assays) or in Medium A supplemented with 5% heat-inactivated (30 min at 56 °C) fetal calf serum (FCS), 5 × 10^{-5} M 2-mercaptoethanol and 2 mM L-glutamine (when used for in vitro experimental assays). The viable cell number is determined by counting in a hemocytometer (see below).