SEARCH FOR POTENT IMMUNOMODULATORY AGENTS FROM PLANTS AND OTHER SOURCES

H. WAGNER
Institute of Pharmaceutical Biology, University of Munich
Karlstrasse 29, D-80333 Munich, Germany

1. Introduction

Immunostimulants are leading predominantly to a nonspecific stimulation of immunological defence mechanisms. Nonspecific stimulants do not affect immunological memory cells and since their pharmacological efficacy fades comparatively quickly, they have to be administered either at intervals or continuously [1,2]. Different are the immunoadjuvants, i.e. complete or incomplete Freund’s adjuvant, which are added to antigens (vaccines) and hereby increase the production of antibodies without acting as antigens themselves [3].

Why do we need immunostimulants? The major concern for developing and applying immunostimulants must be directed to restore the chronically suppressed immune system. The immunosuppression can be caused by bacterial and viral infections, cancer, environmental agents (i.e. pesticides or allergenes), excessive chemo- or radiotherapy, malnutrition or psychic stress. There is a concurrent under the experts that an effectively working immune system is a requirement for full protection against infections and the various sequels. In particular, immunostimulants are an attractive alternative to conventional chemotherapy when mixed infections, infectious hospitalism, chronic infectious diseases, persistent infections and resistant bacterial or viral infections have to be treated.

2. Screening methods

The result of more than 15 years of screening experience in the author’s laboratory has shown that the best short-cut to find effective immunostimulants in plants are investigations using experimental animal models. The infectious stress or immunosuppression models have first preference. They indicate whether or not, and to what extent a drug is able to antagonize a severe, or otherwise lethal infection, and hereby show the protective or therapeutic potential of a drug. The immunosuppression model reveals to what extent a drug under test is able to restore an impaired or unbalanced immune system.

For mass screening, however, the various in vitro bioassays are the adequate meth-
ods, since at the same time they provide hints on the possible mechanism of action of a drug [4]. Since the results obtained in vitro not necessarily must have a counterpart in vivo, it is necessary to confirm them by in vivo experiments. Vice versa it is possible that positive in vivo results cannot be corroborated in an in vitro assay, when several cooperating cells or mediator systems nonpresent in an in vitro cell system are responsible for the in vivo effect.

In general, since there is no master or key cell in the immune system which governs and regulates all various immune reactions it is often necessary to carry out several in vitro assays.

2.1 IN VIVO ASSAYS

2.1.1 Infectious stress assays with mice [5,6]
These assays evaluate whether, and to what extent, a claimed immunostimulating agent is able to protect mice against an otherwise lethal infection.

The drug to be tested is administered i.v. to mice (day -1), 24 hrs (day 0) the animals receive an i.v. injection of a pathogenic microorganism (i.e. Candida albicans, Listeria monocytogenes). A second injection of the immunostimulant is administered on day +1. The protective effect can be measured by either

- a follow-up registration of the survival or death rate after a defined time,
- a continuous estimation of blood parameters (counts of leukocytes, T- and B-lymphocytes etc.) of pretreated and infected mice,
- removal of spleen, liver or kidney from mice, homogenization and transfer of an aliquot amount to agar plates and counting the number of CFU (colony forming units).

Note: Listeria monocytogenes infections are mainly macrophage dependent whereas the infection load with Candida is primarily controlled by granulocytes.

The above described investigations can be carried out with mice immunocompromized by treatment with cyclosporin A, cyclophosphamide or radiation. This treatment suppresses the function of the T-lymphocyte system.

2.1.2 In vivo phagocytosis assay [7]
This test is carried out with mice. The substance under test is administered i.p. or orally to mice. After 24 hrs each mouse receives an i.v. injection of 0.3 ml of Indian ink dispersion (colloidal carbon particle/30g bodyweight). Blood samples are taken from the retro-orbital venous plexus at intervals of 3, 6, 9, 12 and 15 min. after i.v. injection. The carbon clearance, i.e. the rate of elimination of the carbon from the blood, is determined by turbidimetric spectrophotometry at 650 nm. Density reading, plotted against time on a logarithmic scale, gives the regression lines. The stimulation rate is obtained as the ratio of the mean regression coefficient of the substance (R̅ar) to the regression coefficient of the control (R̅cr).