IN VITRO EVALUATION OF MACROPHAGE MEDIATED HOST DEFENSES
AGAINST NEOPLASTIC DISEASE

R. Kirsh¹,² and P. J. Bugelski³
Departments of Drug Delivery² and Experimental Pathology³
SmithKline & French Laboratories
King of Prussia, Pa. 19406

Introduction

The ability of malignant tumors to disseminate to organs distant from the site of the primary lesions is the principal reason for failure in cancer therapy.¹ There are several reasons for this high failure rate. First, at the time of initial diagnosis, metastatic lesions may be too small to detect using currently available diagnostic methodologies; second, the anatomic location of the metastatic lesion may limit the dose of antineoplastic drug that can reach the site without excessive toxicity; and third, the heterogeneity of tumor cell phenotypes within a single lesion is sufficiently diverse, that the responsiveness of cells from one lesion to an antineoplastic modality can differ from both the primary lesion and other metastases.²-⁴ This latter issue suggests that the development of an effective therapy for metastatic disease approaches that will circumvent the problems of cellular heterogeneity.

Within the past several years, considerable information has been obtained indicating that cells of the mononuclear phagocyte series play a major role in the recognition and destruction of tumor cells in situ and that amplification of macrophage-mediated tumoricidal activity could offer a potentially useful modality for the therapeutic management of cancer, including metastatic disease.⁵-⁸

Macrophages rendered tumoricidal through interaction with a variety of synthetic or naturally occurring agents acquire the capability of selectively recognizing tumor cells regardless of their degree of phenotypic diversity.⁹ In addition, macrophage mediated tumoricidal activity appears to be devoid of the problems of generation of resistance which is often observed with cytotoxic drug therapy and specific immunotherapeutic modalities.¹⁰ For these reasons, a significant effort is currently being undertaken in both academic and industrial laboratories to identify novel agents that selectively enhance macrophage-mediated tumoricidal activity in vivo.¹⁰

In this article, we will discuss the methodologies involved in the culture of rodent macrophages and human monocytes; activation to the tumoricidal state, and evaluation of macrophage-mediated tumoricidal activity in vitro.

In Vitro Culture Of Rodent And Human Mononuclear Phagocytes

Preparation of Murine and Rat Peritoneal Macrophages: Specific pathogen-free mice or rats are injected intraperitoneally (i.p.) with 2 ml or 5 ml thioglycollate broth respectively, and killed (ether euthanasia) 5 days later. The animals are swabbed with iodine solution and with 70% ethanol, and pinned to a dissecting board. The abdominal skin is reflected and 5 ml sterile Ca²⁺,Mg²⁺-free Hanks' BSS (HBSS) is injected i.p. (18 gauge needle). Following gentle massage, the peritoneal fluids are aspirated (18 gauge needle) and the cell suspension is centrifuged (600 x g, 5 min) and
Resuspended in serum-free Eagle's DMEM. The cells are plated into 120-mm culture dishes (10^7 cells per dish) and the cultures washed 40 min later, to remove all nonadherent cells. Rapid adherence to glass or plastic in serum-free medium is selective for macrophages. The adherent macrophages are gently scraped off with a rubber policeman.

**Rodent Alveolar Macrophages:** Experimental animals are anesthetized by i.p. injection of sodium pentobarbital, thorax and abdomen swabbed with iodine solution and 80% ethanol. Skin covering the thorax is carefully reflected and the salivary gland removed exposing the trachea.

Carefully insert syringe needle into the trachea at the cricoid cartilage and lavage lungs with prewarmed HBSS using 5 ml/rat and 1 ml/mouse. Following lavage step, alveolar macrophages should be purified as described for peritoneal macrophages. Yield of alveolar macrophages should be approximately 5 x 10^6/rat and 3 x 10^5/mouse.

**Human peripheral blood monocytes:** Peripheral blood monocytes are isolated from one day old platelet pheresis residues obtained from the Red Cross. Briefly, residues are washed with HBSS and mononuclear cells (lymphocytes and monocytes) are isolated by density gradient centrifugation on Ficoll-Hypaque (Lymphoprep® Accurate Chemical Co). The mononuclear cell fraction collected at the interface is further subfractionated into lymphocytes and monocytes by centrifugation over isosmotic 46% Percoll (Pharmacia Inc., Upsalla) at 550 x g for 45 minutes. Monocytes are collected at the interface. The purified monocyte fraction is then washed two times with HBSS and concentration adjusted to 3-4 x 10^6 cells/ml. Following this method, monocyte purity is routinely greater than 95% based on morphology and phagocytic activity.

**II. STRATEGIES FOR MACROPHAGE ACTIVATION IN CANCER THERAPY**

**A. The Activated Macrophage**

Considerable attention has been devoted to the role of activated macrophages in host defense against invading bacteria, parasites, and tumor cells. The term "activated macrophage" is an operational definition and is used differently by various authors to describe acquisition of a variety of functions that are not exhibited by resident tissue macrophages. Examples of the use of the term activation can be found referring to activated macrophages as cells that show oxidative metabolism but lack microbicidal or tumoricidal activity, whereas other authors, including ourselves, limit the use of the term to macrophages that exhibit both microbicidal and tumoricidal properties. Acquisition of tumoricidal properties may be accompanied by other phenotypic alterations including increased phagocytosis, secretion of neutral proteases and acid hydrolases, synthesis and release of arachadonic acid metabolites, expression of an altered ectoenzyme profile, the ability to suppress natural killer (NK)-cell activation, and an enhanced ability to kill intracellular microorganisms. In this review, macrophages that display a biochemical and/or physiological profile different from resident tissue macrophages, but which do not express tumoricidal activity, will be referred to as stimulated macrophages.

Despite this useful general definition, the term "macrophage activation" is often the source of a great deal of confusion and misunderstanding. Of the many reasons for this confusion, the following deserve emphasis:

- Macrophages have on their surfaces at least 30 distinct receptors and are capable of the secretion of over 75 different substances. The expression or production of most of these is alterable by stimulation or activation. Preoccupation with the measurement of many of these parameters after the in vitro stimulation of macrophages with defined stimuli does not reflect what is likely to be a complex regulatory process in vivo.

- No definitive evidence exists for the presence of macrophage populations with distinct differentiation patterns. Despite this fact, macrophage populations are quite heterogeneous in regard to expression of various phenotypic and functional markers. (20,21)

- Significant differences exist in regard to phenotypic and functional parameters of macrophage activation among different species and even among different strains within a species. (16,22-24) Most distressing is the observation that without standardization of animal vendors, mouse...