Effects of ‘Bordetella pertussis’ on Hemic Colony-Forming Cells and the Immune Response\(^1\)

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Abstract

Single injections of ‘Bordetella pertussis‘ were administered to BDF\(_1\) mice, and levels of colony forming cells (CFC) in bone marrow and spleen were determined at selected time intervals after injection. Our studies demonstrated that such injections have multiphasic effects on the levels of CFC present in bone marrow and spleen. To test whether the immune response was altered by ‘B. pertussis’, cultured spleen cells from treated mice were measured for their ability to produce plaque forming cells (PFC), in vitro, and to participate in mixed cell interactions, in vitro. Augmentation or suppression of the immune response in vitro depended on the inoculum size of cultured cells as well as the time of assay after injection.

Introduction

Injection of a variety of antigens, endotoxins, adjuvants, and polynucleotides resulted in alterations in the levels of precursor cells of the hemopoietic renewal system [1-5]. Many of these same agents can also alter the immune response. They can enhance or depress the immune response depending on the timing of injection of either antigen or agent [6, 7]; non-specific enhancement of tumor immunity (immunopotentiators) has also been shown for some of the agents [8-12].

The aim of this study was to evaluate the effects of *Bordetella pertussis* injections into mice on hemopoietic toxicity and, concomitantly, on the development of the immune response. As indicated by FLOERSHEIM [13], the limiting factor in effective use of agents active in both cancer chemotherapy and suppression of the immune response is non-specific cytotoxicity; the bone marrow is usually the dose-limiting tissue. We extended this study to include in vitro assays which analyze precursor cells of granulocytic-monocytic cells (CFC) and spleen cells which develop, in vitro, into antibody forming cells (AFC).

We used the colony forming cell (CFC) assay [14] to determine effects of *Bordetella pertussis* on hemopoietic precursor cells. To determine whether development of AFC was altered, spleen cells from treated animals were cultured, in vitro, to produce plaque forming cells (PFC). Single injections of *Bordetella pertussis* were administered to BDF\(_1\) mice, and CFC and PFC assays were performed at selected time intervals after injection.

Materials and methods

(1) Animals

Female BDF\(_1\) mice (C57B1/6 × DBA/2), 8-12 weeks old, were purchased from Jackson Laboratories, Bar Harbor, Maine; female CFW, 8-12 weeks old, were obtained from Carworth Red Lion, Vincentown, New Jersey.

(2) Tissue culture media

Fetal calf serum was obtained from Reheis Chemical Co., Kankakee, Ill., and sheep red blood cells (SRBC) were obtained from Mogul Diagnostics, Madison, Wisconsin. Concentrates of minimum Eagle’s medium (MEM), medium NCTC 135, horse serum, and guinea-pig complement were obtained from Grand Island Biological Co., Grand Island, N.Y.

(3) Injection

BDF\(_1\) mice were injected intraperitoneally with 0.2 ml of *Bordetella pertussis* vaccine (Eli Lilly, Lot No. 4TWOO). Mice were killed (in groups of three) at 1, 4, 7 and 14 days after injection. At these time intervals cell suspensions were prepared from spleen and bone marrow.

(4) Bone marrow culture technique

All cultures were performed in 35 mm Falcon plastic dishes.
tissue culture (Falcon Plastics, Los Angeles, Calif.). A modification of the technique of METCALF and MOORE [14] was used for culturing colony-forming cells (CFC) in vitro. Briefly, this involves extrusion of bone marrow from femurs and gentle trituration of marrow plugs to obtain dispersed bone marrow cell suspensions. When spleen cells were used, spleens were teased in culture medium with fine toothed forceps, gravity sedimented for 3 minutes to remove cell clumps, and the supernatant was centrifuged to collect the dispersed spleen cells. Using NCTC 135 tissue culture media supplemented with l-asparagine, 10% fetal calf serum and 5% horse serum, 1 x 10^5 bone marrow or 2 x 10^6 spleen cells were cultured in 1.0 ml of semi-solid agar (final concentration was 0.3% of Bactoagar) containing colony stimulating factor (CSF). CSF used for these studies was obtained either from sera of Salmonella antigen (Type H) injected mice, or from conditioned media of L-cell cultures. The cultures were placed in humidified air-tight boxes and briefly gassed with a 12% CO₂-8% O₂-80% nitrogen gas mixture. After seven days of incubation at 37 °C, culture dishes were examined for colony formation at X 20 magnification using an inverted Unitron Microscope.

(5) Development of PFC, in vitro

In this study immune potential is defined as the ability of cultured spleen cells, in vitro, to produce PFC or to participate in allogeneic cell interactions; both of these assays can be performed with aliquots of the same cell suspension, hence facilitating comparison of the effects of pertussis injections on humoral and cell-mediated reactions. In vitro culture of PFC: Cell culture conditions for production of PFC, in vitro, were described by MISHELL and DUTTON [15]. Details of the procedure used to assay PFC were reported in an earlier study [16]. As a routine procedure, 5 to 20 x 10^6 spleen cells were plated with SRBC in 1.0 ml of MEM containing 10% fetal calf serum and cultures were assessed for PFC on the fourth day of cultivation.

(6) Mixed cell interaction coupled to PFC culture, in vitro

In vitro production of PFC was used as an indicator of allogeneic cell interaction [17]. 3 x 10^6 control or B. pertussis-treated spleen cells (BDF1) were mixed with 12 x 10^6 CFW spleen cells; separate cultures containing cells without mixing served as controls. After four days of incubation, cultures were assayed for PFC.

Results

(1) Bone marrow and spleen CFC

A tetraphasic curve for bone marrow CFC was obtained in response to Bordetella pertussis injection (Fig. 1). Bone marrow CFC decreased to low levels (32% of controls) by the first day. On day 4, marrow CFC attained control values. By day 7, marrow CFC dropped again to values which were 33% of controls; 14 days after B. pertussis injection, marrow CFC levels were elevated significantly above control levels (175% of controls). Since the total nucleated femoral cell counts (Table) did not differ significantly at the measured intervals (between 0.74 and 0.97 x 10^7 per femur), the total CFC/femur reflects the fluctuations observed on 10^5 spleen cells.

Less than 2 CFC/10^5 spleen cells cultured