EFFECTS OF *TRICHINELLA* SOLUBLE ANTIGENS ON MACROPHAGE SUBPOPULATIONS

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The immunomodulatory effects of *Trichinella spiralis* or *Trichinella pseudospiralis* soluble antigen extracts were examined in an effort to characterize the differences in immune responses seen during these *Trichinella* infections. Peritoneal cells and fluid recovered from control and infected mice were used during this study. The total number of peritoneal cells recovered during both infections reached a maximum at day 12 PI. Significantly greater numbers of peritoneal macrophages were recovered during *T. pseudospiralis* infections on day 12 PI, than were recovered on day 12 PI during *T. spiralis* infections. Peritoneal macrophages recovered during *T. spiralis* or *T. pseudospiralis* infections were labelled by indirect immunofluorescence with the rat monoclonal antibodies M1/70 (α-Mac-1), M3/38 (α-Mac-2), and B21-2 (α-$Ia^{b,d}$). The predominant phenotype expressed by peritoneal macrophages during these infections was Mac-1^{+}, Mac-2^{-}, and Ia^{+}. Peritoneal fluid was recovered from mice during these two infections and assayed for prostaglandin E (PGE) content. A higher level of PGE in peritoneal fluid occured on day 4 PI during *T. pseudospiralis* infections, than was seen on day 4 PI during *T. spiralis* infections. Peritoneal macrophages recovered from untreated or BCG cell wall-primed mice were cultured with different doses of *T. spiralis* or *T. pseudospiralis* adult, newborn larvae, and muscle larvae antigen extracts. Newborn larvae extracts of either parasite had the greatest potency for stimulating macrophage PGE production, followed

by the adult extracts, and muscle larvae extracts were the lowest. The adult antigen extracts of *T. pseudospiralis* induced significantly greater PGE production by peritoneal macrophages recovered from untreated and BCG cell wall-primed mice, than did the adult antigen extracts of *T. spiralis*. The newborn larvae extracts of either parasite exhibited similar potency for stimulating macrophage PGE production; however, the muscle larvae extracts of *T. pseudospiralis* stimulated greater levels of PGE than did the muscle larvae extracts of *T. spiralis*. These data clearly indicate that *Trichinella* antigens possess immunomodulatory capabilities. Furthermore, these data demonstrate that differences exist between *T. pseudospiralis* and *T. spiralis* in their ability to induce PGE production.

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CHAPTER I

INTRODUCTION

Parasites of the genus *Trichinella* have evolved a distinct sylvatic life cycle which can be transmitted into domestic animal and human populations (Zimmermann, 1972). Until recently, the incidence of trichinosis in this country was significant; however, with the advent of federal inspections and regulations governing domestic meat production the occurrence of trichinosis has decreased. In spite of this vigilance, occasional outbreaks occur from human consumption of wild game and uninspected meats. Thus trichinosis is a continuing focus of public health concern.

The life cycle of these parasites has been thoroughly studied in a number of animal models, and knowledge about these parasites is extensive. However, explicit information concerning details of immunological and physiological changes that occur in the host during the infectious process are few. Host responses to infection with *T. spiralis* are able to diminish, but not to prevent, reproduction of adult worms whose larval progeny ultimately gain access to host striated muscle fibers and encyst. The basis of the host response in primary infections is an immunological reaction that begins to affect the parasite about 7 days following ingestion of the contaminated meat. Within 24 to 48 hours after the end of the first week the host begins to expel the adult worms from their location in the small intestine.

The life cycles of the geographic isolates of *Trichinella* are similar, and the different stages in the life cycle of these parasites are accomplished in one definitive host. When a prospective host ingests trichinous meat, the infective L_1 larvae are released from collagenous capsules in the muscle fibers by the action of host gastric juices. The freed larvae enter into the small intestine and subsequently penetrate the intestinal mucosa to take up residence as an intramulticellular parasite within enterocytes of the host small bowel (Gursh, 1949; Despommier et al., 1978). Shortly thereafter copulation occurs between adult male and female worms. After embryogenesis, females release newborn larvae which gain access to host lymphatics and subsequently enter the circulatory system. These migratory larvae eventually seek out and penetrate host striated myofibers and dramatically alter the biochemistry and ultrastructure of the muscle cell. The life cycle of the parasite is completed approximately 30 days later when larvae in the muscles are enclosed within a collagenous capsule. The encapsulated larvae are thus ready to infect a new host.

Rodents infected with *T. spiralis* develop a significant immune response. In mice, the host response is most effective during the intestinal phase of the infection, therefore limiting the number of migratory larvae invading muscle tissue by reducing fecundity in adult worms and inducing expulsion of adult worms from the intestinal mucosa. Most investigators believe that parasite antigens released during the intestinal phase induce a host reaction which culminates in an allergic inflammation in the area occupied by the adult worms (Larsh and Race, 1975). The inflammatory event is characterized by: (1) A rapidly developing acute reaction identified by an infiltration, into the site, of large

numbers of polymorphonuclear leucocytes, with associated mucosal edema, which is at a zenith about 9 days after infection, and (2) a chronic inflammatory infiltrate consisting of a mixture of plasmocytes, lymphocytes, and macrophages that occurs around day 10 of the infection (Larsh and Race, 1954). Inflammation induced changes in the entero-environment causes the eventual expulsion of adult worms (Castro et al., 1973).

A plethora of cellular and humoral mechanisms are involved in the immune response which ultimately contributes to the expulsion of the adult worms from the host. Antibodies directed against worm antigens are capable of enhancing worm expulsion and reducing adult female fecundity (Despommier et al., 1977; Wakelin and Wilson, 1980; Gamble, 1985). Proliferation of B cells occurs in the gut-associated and peripheral lymphoid tissues (Ljungstrom and Huldt, 1977; Jones et al., 1984). Although B cells and antibodies appear related to the inflammatory events underlying expulsion, the initiation and progression of the inflammatory response are orchestrated almost entirely by thymus-derived (T) lymphocytes. Evidence for the crucial role of T cells in this phase of the host response is provided by studies on thymus-deficient (Ruitenberg and Steerenberg, 1974; Perudet-Badoux et al., 1980), thymus deprived (Walls et al., 1973), and T cell deficient hosts (Ljungstom and Ruitenberg, 1976). Furthermore, L3T4$^+$ T helper cells have been implicated in the adoptive transfer of immunity to non-immune hosts (Wakelin and Wilson, 1979; Grenecis and Wakelin, 1982; Wakelin and Denham, 1983; Grenecis et al., 1985), and this subpopulation of T cells is responsible for inducing antibody and lymphokine production. These T helper cells are essential to host immune

responses, because they mediate lymphocyte proliferation and the effector functions of most immune cells.

The role of *Trichinella* surface and secreted antigens in host immune responses have been examined in numerous investigations. The muscle stage larva of *T. spiralis* is the focus of most of these investigations. Monoclonal antibodies raised against muscle larvae antigens are used to identify localized sites for antigen synthesis, such as the polypeptides of 48 and 50/55 kDa which are found in the β and α stichocytes respectively, as well as the cuticle and gut lining (Silberstein and Despommier, 1984). A number of research groups have characterized surface and excretory/secretory antigens that provide protection in mice against challenge infection (Gamble, 1985a; Silberstein and Despommier, 1984; Zhu and Bell, 1990). Furthermore, their appears to be differences in the immunogenicity of stage derived antigens (Gamble, 1985b; Marti et al., 1987).

The chemical composition of *Trichinella* species are different as determined by the biochemical analysis of antigens. Stage specific differences exist between *T. spiralis* and *T. pseudospiralis*. Stage specific profiles of surface labelled proteins derived from newborn larvae are similar, whereas profiles derived from adults and infective larvae are different (Almond et al., 1986). Likewise, another research group found compositional differences in crude muscle larvae antigen and surface detergent stripped antigens from *T. spiralis* and *T. pseudospiralis* (Bolas-Fernandez and Wakelin, 1990). Furthermore, these compositional differences may account for differences in host response when related to the biology of the two worms (Palmas et al., 1985). Since constitutive differences exist between the

surface and internal antigens of *T. spiralis* and *T. pseudospiralis* , it is likely that host immune responses to *T. spiralis* and *T. pseudospiralis* antigens are different.

With regard to the occurrence of inflammation in host tissues during *Trichinella* infections, investigators have recently discovered that host response to infection with *T. pseudospiralis* differs markedly from that seen in *T. spiralis* infections. *Trichinella pseudospiralis* differs from *T. spiralis* in both morphology and the host parasite relationship. *T. pseudospiralis* is smaller at all stages, is less fecund, and the infective larvae lack a collagenous capsule that encloses this stage in the *T. spiralis* life cycle (Stewart, 1989). Although there is no definitive knowledge on the specific mechanisms involved in host responses to *T. pseudospiralis*, it is known that these responses differ markedly in intensity and the nature of the cells involved from those seen during infection with *T. spiralis*. Mice infected with *T. pseudospiralis* develop significantly lower levels of enteritis (Kramer et al., 1981) and myositis (Stewart et al., 1985), and also exhibit a reduced inflammatory response to experimentally introduced stimuli such as subcutaneously implanted cotton string (Stewart et al., 1985). Moreover, during concurrent infections in mice with *T. spiralis* and *T. pseudospiralis*, the influx of inflammatory cells is dramatically suppressed (Stewart et al., 1985; 1988). Also the natural resistence of the chinese hamster to *Trichinella* infection is overcome when these animals are infected with *T. pseudospiralis* (Stewart and Larsen, 1989). Furthermore, *T. pseudospiralis* muscle larvae may evade host immune defenses by inducing corticosteroids (Stewart et al., 1988) and by expressing the host-like antigen asialo GM1 (Niederkorn, 1988). These altered levels of host inflammatory response suggest that *T. pseudospiralis* is able to modulate host immune function in a

manner which seems to be beneficial to the parasite. It is likely that such modulation is accomplished by parasite induced alterations in host mechanisms that normally exercise control over the immune response.

The host cell type that may have the most significant effect on the progression of inflammation is the macrophage. Macrophage activity is enhanced (Meerovitch and Bomford, 1976) and their numbers increase in and around the tissue sites occupied by *T. spiralis* (Larsh and Race, 1954; Komondarev et al., 1977). Recent investigations have revealed that the macrophage produces interleukin-1 (IL-1) and prostaglandin E_2 (PGE_2) which are able to regulate a large number of functions in both the immune response and inflammatory process (Whicher and Chambers, 1984; Oppenheim et. al.,1986).

Suppression of immune cell function in mammals is typically mediated by T cells and macrophages via release of soluble mediators such as cytokines and metabolites of arachidonic acid. The arachidonic acid metabolite PGE_2 is released by macrophages and has a negative regulatory role in immune/inflammatory responses (Stenson and Parker, 1980; Oppenheim et al., 1986). Parasite factors may contribute to a suppression of host cell function either directly via factors such as taeniaestatin (Leid et al., 1986) or indirectly by stimulating the host to produce high levels of glucocorticoids (Ranelletti et al., 1986; Reed et al., 1986). All of these factors have been shown to block lymphocyte proliferation and lymphokine secretion, thus effectively reducing an immune response.

The regulatory mechanisms behind the suppression of host response during infection with *T. pseudospiralis* are presently not well known. However, recent observations support the hypothesis that alterations in macrophage functions during the

intestinal phase of *T. pseudospiralis* infections in mice, may account for some of the immunosuppression seen during this infection. Possible explanations for the reductions in host response seen in *T. pseudospiralis*-infected mice might be alterations in macrophage accessory functions; antigen processing and antigen presentation, Ia antigen expression, and IL-1 production. Likewise, macrophage suppressor factors such as prostaglandin E_2 (PGE_2) may be dampening host response to *T. pseudospiralis* infection.

Surface proteins associated with immune cells are of critical importance in the establishment and maintenance of an immune response to antigenic substances. Historically, immune cell surface proteins were used as markers to delineate individual immune cell populations such as T cells, B cells, and macrophages. More recently researchers began revealing subpopulations of immune cells based on the expression or lack of surface markers that were then correlated with functional properties. These surface markers are also used to distinguish different ontogenetic states of immune cells. Surface markers are invaluable research tools in characterizing the immune response as it relates to the well being of man and animals.

Since macrophages are important immune mediator cells, numerous investigations have revealed that macrophages serve an accessory function in humoral and cell-mediated immunity. Further observation of these cells demonstrated their ability to mediate many facets of the inflammatory response. The principal surface markers studied during the aforementioned investigations are the macrophage I-region associated (Ia) antigens, immunoglobin Fc receptor (FcR), and complement C3b receptor (CR3). The presence of macrophage Ia markers (class II MHC antigens) is used to assign

accessory or antigen presenting function to Ia bearing cells (Beller et al., 1980; Beller and Unanue, 1981; Vogel et al., 1983; Unanue et al., 1984; Kohno et al., 1987). The level of FcR and CR3 expression on macrophages is used to define activation states primarily associated with inflammation and infection (Beller et al.,1980; Vogel et al., 1983). These macrophage surface markers and others define functionally diverse macrophage populations. Monoclonal antibody technology allows precise measurements of surface markers on cells via immunocytochemical and immunohistochemical techniques. Rat hybridomas that secrete monoclonal antibody (Mab) against murine macrophage surface markers are widely available. Recently, three macrophage surface markers were characterized with the aid of their respective rat Mabs. These markers are Mac-1, Mac-2, and Ia. Results from several investigations reveal that these macrophage markers will further delineate macrophage subpopulations (Springer et al., 1979; Steinman et al., 1980; Nussenzweig et al., 1981; Ho and Springer, 1982; Vogel et al., 1983; Garner et al., 1987; Walker, 1987).

Rat Mab M1/70 (anti-Mac-1) immunoprecipitates polypeptides of 190,000 and 105,000 molecular weight (MW) from macrophages as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Springer et al., 1979). Autoradiography demonstrated that Mac-1 selectively labeled macrophages, histiocytes, and to a lesser extent granulocytes; however, lymphoid cells were not labelled (Springer et al., 1979). Binding studies revealed specific binding by the $F(ab')_2$ portion of M1/70 to adherent peritoneal cells (Springer et al., 1979). Recent studies have identified Mac-1 as the presumptive CR3, since anti-Mac-1 blocks C3bi mediated phagocytosis by macrophages (Beller et al., 1980; Beller et al., 1982; Ding et al., 1987).

Rat Mab M3/38 (anti-Mac-2) precipitated a 32,000 MW polypeptide and a lesser amount of a 30,000 MW polypeptide from ^{125}Iodine-labelled peritoneal exudate cells (PEC; Ho and Springer, 1982). Mac-2 antigens did not contain any interchain disulfide bonds, since reducing agents failed to change SDS-PAGE mobilities (Ho and Springer, 1982). Both polypeptides were biosynthetically labelled with [^{35}S]-methionine (Ho and Springer, 1982). Further experiments estimated that there are an average of 1.7×10^5 binding sites per thioglycollate (TG) elicited macrophage (Ho and Springer, 1982). Mac-2 was expressed on TG-elicited PEC and not on lymphoid cells when examined by immunofluorescent flow cytometry (Ho and Springer, 1982).

Rat Mab B21-2 (anti-I-$A^{b,d}$) labelled with ^{125}Iodine bound to spleen cells from mouse strains bearing I-A^b and I-A^d major histocompatibility (MHC) antigens (Steinman et al., 1980). Class II MHC antigens are transmembrane glycoproteins that possess intrachain disulfide bonds and average about 32 kDa in size. B21-2 precipitated polypeptides from spleen cells that are typical of class II MHC polypeptides (Steinman et al., 1980) and these polypeptides had a molecular weight range of 34,000-25,000 (Nussenzweig et al., 1981). B21-2 labelled typical Ia expressing cells including: macrophages, dendritic cells, B cells, and Langerhans cells (Nussenzweig et al., 1981; Schuler and Steinman, 1985).

The major histocompatibility complex (MHC) is a complex genetic region, which encodes at least three families of molecules; class I, class II, and class III. The MHC of the mouse (H-2 complex) maps to chromosome 17 and the MHC of man (HLA complex) maps to chromosome 6. Class I and class II molecules are heterodimeric

cell-surface glycoproteins, whereas class III molecules are elements of the complement system present primarily as serum proteases. Class I molecules are generally referred to as transplantation antigens (murine K, D, L; human A, B, C) and are found on virtually all nucleated cells, thus serving as targets for cytotoxic T cells. The class II molecules are involved in communication between cells that regulate the immune response. Class II antigens are found in the I locus (I-A, I-B, I-J, I-E, I-C; I-associated [Ia] antigens) of the H-2 complex of mice and the HLA-D locus (D-related [DP,DQ,DR] antigens) of man. Furthermore, the polymorphic nature of the class II molecules is readily illustrated by the unique MHC haplotype of the independently derived strains of inbred mice. For example Balb/c mice express a $H\text{-}2^d$ haplotype, whereas C57bl/6 mice express a $H\text{-}2^b$ haplotype.

Class II MHC antigens are heterodimeric glycoproteins that are composed of two chains; a 33-35 kDa heavy (α) chain and a 27-29 kDa light (ß) chain. Amino acid sequences deduced from cloned DNA show that there are four domains: Two extracellular domains of approximately 90 amino acids each, a N-terminal domain, and a C-terminal domain close to the membrane. Each chain is linked to a polypeptide that anchors them in the membrane and extends into the cytoplasm. Both chains have disulfide bonds that form peptide loops. Interestingly, the second domains of both chains (α_2 and $ß_2$) exhibit strong sequence homology to immunoglobin constant regions, and the first domains (α_1 and $ß_1$) are globular (Kaufman et al., 1984). Class II MHC antigens are found on B cells, activated T cells, and antigen presenting cells (APC) such as; dendritic cells, tissue macrophages, and Langerhans cells.

Antigen presentation is the complex mechanism necessary for the recognition and stimulation of effector T lymphocytes (CD4 and CD8), the T cell subsets responsible for potentiating host immune responses to foreign proteins, bacteria, parasites, and viruses. The antigenic moiety must first be endocytized, processed, and incorporated into the structure of the class II MHC molecule prior to being presented to T cells (Harding and Unanue,1989; Unanue and Cerottini, 1989). The macrophage is the most ubiquitous APC found in tissues.

Macrophage expression of Ia indicates a functional state and probably a specific developmental stage of the monocyte/macrophage lineage (Beller et al., 1980; Beller and Unanue, 1981). Lymphokines such as τ-interferon and IL-4 up-regulate the expression of Ia by mouse macrophages (Vogel et al., 1982; Steinman, 1988; Stuart et al., 1988; Cao et al., 1989). These lymphokines probably serve as an early signal in a progressive sequence of macrophage differentiation. Other immune/inflammatory mediators such as eicosanoids and IL-1 also influence Ia expression and APC function. Eicosanoids such as E series prostaglandins negatively regulate Ia antigen expression by dendritic cells and peritoneal macrophages (Unanue et al., 1984). Conversely, IL-1 augments APC function in initiating T cell antigen recognition and eventual T cell proliferation (Kohno et al., 1987; Steinman, 1988). Thus, Ia expression and APC function is dependent on sequential events and is differentially regulated by cytokines and prostaglandin E.

Mac-1 protein belongs to a family of adhesion-related glycoproteins termed $ß_2$ integrins. Current nomenclature identifies Mac-1 as the cluster designation (CD) 11b. All $ß_2$ integrins belong to the CD11\CD18 complex of leukocyte adhesion proteins. $ß_2$

integrins consist of an α subunit of 150-190 kDa and a ß subunit of 94 kDa (Arnaout, 1990). The divalent cations Ca^{2+} and Mg^{2+} are essential in the stabilization and function of the $\alpha_1ß_1$ heterodimeric complex of the $ß_2$ integrins (Arnaout, 1990). Recently the $ß_2$ integrin α subunits were mapped to the short arm of human chromosome 16 and the ß subunits were mapped to chromosome 21 (Marlin et al., 1986; Corbi et al., 1988). Mac-1 is apparently a multispecific receptor for a diverse group of ligands. Mac-1 binds: C3bi (Beller et al., 1982; Aranout et al., 1983; Wright et al., 1983), LPS in the outer leaflet of the bacterial membrane (Wright and Jong, 1986), Factor X and fibrinogen (Altieri et al., 1988), and *Leishmania* advanced glcosylation products (Mosser et al., 1987). Mac-1 also is an integral component in antibody mediated killing of *Shistosoma mansoni* shistosomula (Vignal et al., 1990). Furthermore, Mac-1 has a substantial role in complement and Ig mediated phagocytosis of microbes (Graham et al., 1989). The cellular dynamics of Mac-1 expression indicate that this marker appears on immature macrophages such as peripheral blood monocytes and a population of bone marrow cells (Springer, et al., 1979). Mac-1 protein expression is up-regulated when blood monocytes enter the tissues and differentiate into macrophages (Springer et al., 1979). Cytokines such as τ-interferon and α-TNF differentially regulate Mac-1 expression (Ding et al., 1987; Pichyangkul et al., 1988; Martin et al., 1988), thus indicating that Mac-1^+ macrophages are activated to progress to a more differentiated state. Furthermore, when cells expressing Mac-1 are stimulated with τ-interferon they decrease Mac 1 expression and up-regulate Ia antigen expression (Vogel et al., 1983). This indicates that upon interferon stimulation macrophages possessing Mac-1 markers progress to a more highly differentiated state. These

data suggest that Mac-1 positive cells possess functions associated with the reticuloendothelial system, and that these cells demonstrate a loss of Mac-1 markers after cytokine stimulation, thus indicating a early developmental state.

Studies involving other Mac proteins have also supported the evidence for diverse macrophage subpopulations. When PEC are collected from mice injected i.p. with TG, the Mac-2 antigen is expressed in greater amounts than Mac-1 antigen (Ho and Springer, 1982; 1983). Other investigations further delineate the nature of Mac-2 expressing cells. Cells possessing Mac-2 antigens are associated with immunosuppressive activity (Garner et al., 1987). Mac-2 positive cells secrete more PGE_2, which mediates suppression of tumor rejection and mixed leukocyte reaction in tumor bearing mice (Garner et al., 1987). These data indicate that Mac-2 positive macrophages serve as immunoregulators during strong inflammatory responses.

A common feature shared by all Mac proteins is their lack of expression on lymphoid cells. This is understandable given the distinctly different functions that macrophages and lymphocytes serve in the immune response. However, contrasts of Mac positive macrophage subpopulations indicate that there is great diversity in expression of these markers, along with divergent functions associated with individual Mac positive subpopulations (Garner et al., 1987; Walker, 1987). Evidence clearly indicates that Mac-1 positive cells are in a earlier ontogenetic state than are Mac-2 expressing cells. Mac-2 positive cells apparently possess regulatory and highly specialized functions. Furthermore, the variability in expression of Mac-1 and Mac-2 markers in these macrophage subpopulations, denotes that subpopulations expressing these phenotypes are heteroge-

nous in function. Mac surface proteins are clearly useful in identifying and delineating macrophage subpopulations. Even more important is the potential for characterizing the role of macrophages in parasitic, immunosuppressive, and autoimmune diseases.

The present study was undertaken in order to clarify the role of macrophages in *T. spiralis* and *T. pseudospiralis*-infected mice. The dynamics of peritoneal cells was evaluated by using total and differential counts. Likewise, monoclonal antibodies were employed to identify and enumerate peritoneal macrophage subpopulations during these infections. Furthermore, the immunomodulatory capabilities of *Trichinella* adult, newborn larvae, and muscle larvae antigens were assessed by assaying macrophage PGE production.

CHAPTER II

MATERIALS AND METHODS

Animals

Six to 8-wk-old male Balb/c inbred mice (Harlan Sprague Dawley, Indianapolis, Indiana) were used in all experiments. Animals were maintained as previously described (Stewart et al., 1985).

Parasites

Experimental animals were infected per os with either 500 *T. spiralis* or 500 *T. pseudospiralis*. The strains of the parasite used, method for isolation of *T. spiralis* L_1 larvae, and method used to infect hosts is described in an earlier publication (Stewart et al., 1985). The method of isolation for *T. pseudospiralis* L_1 infective larvae was described in Stewart et al. (1990). The levels of infection used result in similar numbers of adult worms of the two species of parasites in the host small intestine.

Parasite Extracts

Adult worms (48 hr-old), newborn larvae, and muscle larvae of *T. spiralis* and *T. pseudospiralis* were collected by methods described previously (Kilgore et al., 1986). Worms and larvae were harvested and processed aseptically by washing 3 X in sterile saline containing 2% antibiotic/antimycotic, suspended in RPMI 1640 and disrupted by sonication at 5° C followed by 30 strokes in a glass homogenizer on ice. Extracts were

then centrifuged at 3000 x g for 20 min, the supernatant was decanted, and stored at -75° C until needed (all extracts were generously provided by Dr. George Stewart).

Monoclonal Antibody Production and Purification

Rat-mouse hybridomas that produce monoclonal antibodies (Mab) against Mac-1 (M1/70.15.11.5, rat IgG 2b), Mac-2 (M3/38.1.2.8, rat IgG 2a), and I-$A^{b,d}$ (B21-2, rat IgG 1) antigens were purchased from ATCC (Rockville, Maryland) and cultured in RPMI SUPH (RPMI 1640 supplemented with 15% fetal bovine serum, 25 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 μM sodium hypoxanthine, 16 μM thymidine, and 0.05 mg/ml gentamicin sulfate; Microbiological Associates, Walkersville, Maryland). One million cells were seeded into 75 cm^2 triangular shaped flasks (Costar) containing 30 ml RPMI SUPH and maintained in a 37° C, humidified incubator under 5% CO_2. After the cell density reached 1 x 10^6/ml the culture was terminated. The culture supernatants were harvested by centrifugation at 1000 x g for 10 min, decanted and stored at 4° C.

The rat Mab containing tissue culture supernatants were affinity purified with a rabbit anti-rat IgG, H and L chain specific immunoaffinity column (Cappel, Malvern, Pennsylvania) while using a 0.01 M Tris buffered saline pH 8.0 starting buffer, and the rat IgG fractions were eluted from the column with elution medium (EM; ICN Biomedicals, Lisle, Illinois). Approximately 100 ml of culture supernatant was purified in each batch. Individual columns were maintained for each Mab to prevent cross-contamination. Affinity purified rat Mabs in EM were desalted and concentrated in Centricon 10 miniconcentrators (10,000 MW cutoff; Amicon, Danvers, Massachusetts) then filtered with 0.2 μm syringe filters (Costar, Cambridge, Massachusetts) and stored at 4° C. The

batches of each purified rat Mab were combined to produce individual lots of the M1/70, M3/38, and B21-2. An aliquot of each lot was then assayed for protein content, purity, and identity as rat IgG. The purified rat Mab lots were dispensed in 0.5 ml aliquots (1 mg/ml) into 12 x 75 mm polypropylene, capped tubes and stored at -80° C until needed.

Protein Assay

An aliquot of each affinity purified rat Mab lot was assayed to determine the total protein content. The protein content was determined by a colorimetric dye-binding assay (Bio-Rad, Richmond, California). A bovine gamma globin standard (1.66 mg/ml; Bio-Rad) was used to construct a standard curve based on 5 standard dilutions. Appropriate dilutions of standards and samples (100 µl) were transferred to a 96 well microtitre plate and read with an automated plate reader/spectrophotometer at 595 nm. The OD_{595} of standards minus reagent blank was plotted versus the concentration of standards (mg/ml). The protein concentration of the samples was then interpolated from the standard curve.

Radial Immunodiffusion Assay

The level of rat IgG in each lot of Mab was determined by radial immunodiffusion (RID) assays. Commercially prepared and calibrated RID plates and standards were used in all assays (Bioproducts for Science; Indianapolis, Indiana). The appropriate RID plate and standards were used for each rat Mab subisotype; rat IgG2b for M1/70, rat IgG2a for M3/38, and rat IgG1 for B21-2. For each rat subisotype assay three reference dilutions were used for calculating a standard curve: 100% (neat), 60%, and 10% of

reference serum. Approximately 5 µl of the standards and the samples were loaded per well into the appropriate plate. The plate was then tightly closed and stored flat at room temperature for at least 48 hours. After a suitable diffusion time, the precipitate diameters were read with an eye piece using side lighting. The square of the diameters of the precipitation formed by the three standards was plotted versus their protein concentration (mg) using linear graph paper. The unknown sample concentrations were interpolated from the standard curve.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Each lot of rat Mab was analyzed for purity by SDS-PAGE with reducing conditions. The gels were cast using the discontinuous polyacrylamide gel method (Laemmli, 1970). First, separating gels were cast using the Mini-Protean II dual slab casting apparatus (Bio-Rad, Richmond, California). The 12% separating gel monomer solution was prepared with 3.35 ml distilled water, 2.5 ml 1.5 M Tris-HCl (pH 8.8), 100 µl 10% (w/v) SDS stock (room temp.), 4.0 ml acrylamide/bis (30%/2.67%), 50 µl 10% ammonium persulfate, and 5 µl TEMED. The monomer solution was mixed and loaded into the glass plates with a Pasteur pipette to 5 mm below the bottom of the sample wells. Then 0.5 ml distilled water was layered on top of the monomer with a 25 g x 5/8" needle and 3 ml syringe to ensure uniform polymerization of the monomer by excluding air. The monomer was allowed to polymerize overnight at room temperature. Next a 4% stacking gel monomer solution was prepared with 6.1 ml distilled water, 2.5 ml 0.5 M Tris-HCl (pH 6.8), 100 µl 10% (w/v) SDS, 1.3 ml acrylamide/bis (30%/2.67%), 50 µl 10% ammonium persulfate, and 10 µl TEMED. The distilled water was removed from the

top of the separating gel, and a teflon comb for forming the gel wells was placed on top. The stacking gel monomer was mixed by gently swirling, and loaded around the gel well comb with a pasteur pipette. The stacking gel was allowed to stand for 1 hr before removing the teflon comb and adding the samples.

The samples were prepared by diluting the samples (10 μg) at least 1:4 with sample buffer (4.0 ml distilled water, 1.0 ml 0.5 M Tris-HCl [pH 6.8], 0.8 ml glycerol, 1.6 ml 10% [w/v] SDS, 0.4 ml 2-b-mercaptoethanol, and 0.2 ml 0.05% [w/v] bromphenol blue), and heated at 95° C for 5 minutes. Thirty μl of the samples and standards (low molecular weight; Pharmacia, Piscataway, New Jersey) were loaded into the gel wells. Then 300 ml of electrode buffer (0.9 g Tris base, 4.32 g glycine, 0.3 g SDS, and distilled water to make up to 300 ml [pH 8.3]) was added to the Mini-Protean II chamber and the gel was run at 200 volts, constant voltage setting, for 45 minutes. After electrophoresis the gel slabs were removed and stained for 30 min with 0.1% Coomassie blue R-250 in fixative (40% methanol, 10% glacial acetic acid in distilled water). The gel slab was then destained with 40% methanol, 10% glacial acetic acid in distilled water overnight. The gel slab was preserved by drying between cellophane sheets (Bio-Rad Slab Gel Dryer).

Ouchterlony Double Diffusion

Each lot of affinity purified rat Mab was assayed for identity as rat IgG by the Ouchterlony double diffusion method. Commercially prepared plates with two antibody wells each surrounded by five antigen wells were used (MA Bioproducts, Walkersville, Maryland). Twenty-five μl of affinity purified M1/70, M3/38, and B21-2 were placed in the antigen wells of the plate, whereas 25 μl of goat anti-rat IgG (Cappel) was placed in

the center antibody well. The plate was placed in a 37° C humidified incubator for 24 hours. After incubation the gel removed from the plate and washed in a dish containing 150 ml PBS for 72 hr, replacing the PBS every 24 hours. The gel was then fixed, stained, and destained (see SDS-PAGE section) until the precipitate bands were visible. The gel was mounted in cellophane and dried.

Viability and Differential Counts

Peritoneal cell viability was determined by trypan blue dye exclusion. Viability was always >95%. Differential counts were done by placing 1.5 x 10^5 cells on a slide with a cytocen-trifuge (Shandon, PA), staining with Diff-Quick (Baxter Scientific Products, Chicago, IL), and counting 100 cells in each of 3 random fields under oil.

Immunofluorescence

Peritoneal cells (PC) were recovered from mice by peritoneal lavage after euthanasia by CO_2 narcosis. The mice were then injected with 8 ml of cold phosphate buffered saline pH 7.3 with 3 mM EDTA (Sigma) and massaged for two minutes. The lavage fluid was withdrawn with a 10 ml syringe and 23 g needle. Recovered cells were centrifuged at 450 x g for 5 min and resuspended in FMS (Hank's balanced salt solution, 0.5% bovine serum albumin, 3 mM EDTA, and 0.1% sodium azide pH 7.3). Approximately 1-2 x 10^6 PC were dispensed into 12 x 75 mm polystyrene tubes and centrifuged at 450 x g for 5 min. PC were resuspended in 150 µl of appropriately diluted control (10 µg/ml affinity purified rat IgG ; Chemicon, El Segundo, California) or rat Mab and incubated for 30 min. All tubes were then washed twice by adding 1 ml of FMS, centrifuged at 450 x g for 5 min, and the supernatant was decanted. Next 150 µl of diluted 2nd

antibody-FITC conjugate (Rabbit $F(ab')_2$ anti-rat IgG [H and L chain specific]; Cappel) was added to the cell pellet and incubated for 30 min. Then the cells were washed twice and resuspended in 1 ml of FMS. All tubes were kept at 4°C until analysis by flow cytometry.

Flow Cytometry

Fluorescence analysis was conducted using a Coulter EPICS V with an argon laser. Analysis of fluorescence events was performed with the MDADS computer (Coulter Electronics, Hileah, Florida). Prior to data collection electronic gates were set to eliminate debris, damaged cells, and red blood cells during data acquisition. A total of 10,000 fluorescent events were counted from the PC suspensions.

Extract Bioassay

Peritoneal cells (1 x 10^6/well) recovered as above, from untreated or BCG-primed (100 μg BCG cell walls [Ribi Immunochemicals, Hamilton, Montana] emulsified in a 50% sterile saline-50% glycerine solution injected i.p. 6 days prior to experimentation) mice were incubated in RPMI SUP (RPMI 1640 with 10% heat inactivated FBS, 25 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, and 0.05 mg/ml gentamicin sulphate) for two hours in 5% CO_2 at 37° C. Adherent peritoneal cells were prepared by removing the nonadherent cells from the 12-well plates (Costar, Cambridge, Massachusetts) by washing the plates 2 times with warm RPMI SUP and disposing of the nonadherent cells. The remaining adherent cells were >85% macrophages as determined by Mac-1^+ immunofluorescence and differential stain morphology. The macrophage cultures were then incubated 16 hours (5% CO_2, 37° C, 95% relative humidity) with 1 ml RPMI

SUP only, or 1 ml RPMI SUP and worm extract. After incubation the culture supernatants were removed and centrifuged at 1000 x g for 5 min in 12 x 75 mm tubes. The supernatants were decanted and stored at 4° C until the PGE was extracted.

PGE Extraction and Radioimmunoassay

The PGE extraction and radioimmunoassay procedures used in this study were modifications of procedures used previously (Jaffe and Behrman, 1974). Culture supernatants were extracted within 48 hours after they were harvested. First the supernatant pH was lowered with 6 N HCl to pH 4. Then three volumes of ethyl acetate was added to the supernatant, followed by vigorous mixing, and centrifugation at 450 x g for 10 min without braking. The organic phase was removed without disturbing the aqueous phase and dispensed into 12 x 75 mm polypropylene tubes, followed by drying under N_2 gas at 35° C, and resuspended in RIA buffer (PBS with 0.1% bovine gamma globin and 0.1% sodium azide). The sample tubes were then assayed within 3 days after PGE extraction. Working dilutions of RIA antiserum were prepared in RIA buffer (anti-PGE-BSA; ICN Immunochemicals, Irvine, CA) and a fresh solution of radiolabeled PGE_2 (100,000 dpm/ml of ^{125}I-iodotyrosine methyl esterPGE_2; 2200 Ci/mmol; NEN Research Products, Boston, Massachusetts) was prepared in RIA buffer. Then five standard dilutions of PGE_2 (Sigma) were prepared in RIA buffer. Next, 0.1 ml of standards or samples were placed in 12 x 75 mm polypropylene tubes. Then 0.5 ml of the antibody solution was added to the tubes and all the tubes were incubated for 30 min at 4° C. Subsequently, 0.1 ml of radiolabelled PGE_2 solution was added to each tube, and incubated for 60 min at 4° C. Finally, 0.2 ml of dextran-coated charcoal solution (500 mg of activated Norit A

charcoal and 50 mg dextran T70 [Pharmacia] mixed thoroughly in 200 ml RIA buffer) at 4° C was added to the tubes, incubated at 4° C for 10 min, vortexed, and centrifuged at 3000 rpm for 15 minutes. Then 0.25 ml of supernatant was removed from each tube and counted for one minute in a gamma counter. A zero control, blank, and total tube were included with each assay. Assay data from duplicate sample tubes were interpolated from a standard curve: On semi logarithmic graph paper the % bound was plotted versus the log dose of standard. Results are expressed in PGE ng/ml.

Statistics

The significance of differences between means was ascertained using Student's *t*-test. All data expressed as percentage were submitted to arc sine transformation before statistical analysis. P values ≤ 0.05 were considered significant.

CHAPTER III

RESULTS

Monoclonal Antibody Production and Purification

Monoclonal antibodies M1/70 (α-Mac-1), M3/38 (α-Mac-2), and B21-2 (α-I-$A^{b,d}$) were produced and purified as specified in the "Materials" section. Table I lists the total protein and radial immunodiffusion (RID) assay results. A lot of each of the monoclonal antibodies was produced from the listed quantity of culture supernatant. All lots were assayed after affinity purification and processing. The total protein and total rat IgG content was determined by multiplying the total volume (ml) of the combined affinity purified antibody fractions by the assay result (mg). Yield of rat IgG (μg/ml) was calculated by averaging the protein and RID assay results, multiplying by 1000 to convert to μg, and then dividing by the volume of hybridoma culture supernatant processed. Hybridoma M1/70 yielded 8.3 μg of rat IgG per ml of culture supernatant, M3/38 yielded 12.79 μg of rat IgG per ml of culture supernatant, and B21-2 yielded 1.44 μg or rat IgG per ml of culture supernatant. Each batch of hybridoma culture supernatant yielded more purified monoclonal antibody than was required to finish this project.

Each lot of the monoclonal antibodies were analyzed for purity by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The samples were

Table 1

Affinity purified monoclonal antibody fractions were assayed for protein and rat IgG content. A colorimetric protein assay was performed on M1/70 (α-Mac-1), M3/38 (α-Mac-2), and B21-2 (α-I-$A^{b,d}$) purified monoclonal antibody fractions. Likewise, radial immunodiffusion (RID) assays were performed on the above monoclonal antibodies, in order to determine the total rat IgG content. The "total" columns represent the total quantity of protein and rat IgG in the pooled Mab fractions. The yield of rat IgG was determined by averaging the protien and RID result for each monoclonal antibody, then converting to total μg, and then dividing by the volume of culture supernatant processed.

Table I.

Monoclonal Antibody Production Summary

Mab	Protein Assay (mg/ml)	Total Protein (mg)	RID Rat IgG (mg/ml)	Total Rat IgG (mg)	Culture Super. (ml)	Yield Rat IgG (μg/ml)*
M1/70	1.95	5.85	2.20	6.6	750	8.30
M3/38	5.70	17.10	4.52	13.6	1200	12.79
B21-2	0.72	1.23	0.30	0.5	600	1.44

* amount recovered for each ml of culture supernatant processed.

prepared and the gel was run under denaturing and reducing conditions. Figure 1 illustrates the purity of the monoclonal antibodies M1/70, M3/38, M3/84, and B21-2. All monoclonal antibody samples exhibit characteristic heavy and light chain bands with very little detectable non-antibody protein. Lanes 1, 4, 5, and 8 are low molecular weight standards (phosphorylase b, 94k; albumin, 67k; ovalbumin, 43k; carbonic anhydrase, 30k; trypsin inhibitor, 20k; and alpha lactalbumin, 14.4k), whereas lane 2 is M1/70 (α-Mac-1), lane 3 is M3/38 (α-Mac-2), lane 6 is M3/84 (α-Mac-3), and lane 7 is B21-2 (α-I-$A^{b,d}$). Monoclonal antibody M3/84 (α-Mac-3) did not contribute to the findings of this study and was dropped from the antibody panel.

The individual lots of purified monoclonal antibodies were identified as rat IgG by diffusion against affinity purified goat α-rat IgG (Ouchterlony double diffusion method). Positive reactions were identified by the formation of a precipitate in the agarose substrate. Figure 2 illustrates the result of reacting goat α-rat IgG with M1/70, M3/38, and B21-2. Samples from each purified lot of the aforementioned monoclonal antibodies formed a precipitate with goat α-rat IgG, thus indicating the prescence of rat IgG.

Immunofluorescent Labelling of Peritoneal Macrophages

The monoclonal antibodies M1/70 (α- Mac-1), M3/38 (α-Mac-2), and B21-2 (α-I-$A^{b,d}$) were validated through the use of flow cytometry. A dual parameter histogram in three dimensional representation illustrates the size (x-axis; forward angle light scatter [FALS]), fluorescent intensity (y-axis; integrated green fluorescence[IGFL]), and cell

Figure 1

Protein electrophoresis was utilized to demonstrate the purity of the affinity column fractions from hybridoma culture supernatants. SDS-PAGE was run under reducing conditions and molecular weight standards were used. Lanes 1, 4, 5, and 8 are low molecular weight standards (phosphorylase b, 94,000; albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; trypsin inhibitor, 20,100; alpha lactalbumin, 14,400). Lane 2 is M1/70 (α-Mac-1), lane 3 is M3/38 (α-Mac-2), lane 6 is M3/84 (α-Mac-3), and lane 7 is B21-2 (α-I-$A^{b,d}$). Single bands of heavy and light chains indicate the purity of the rat monoclonal antibodies.

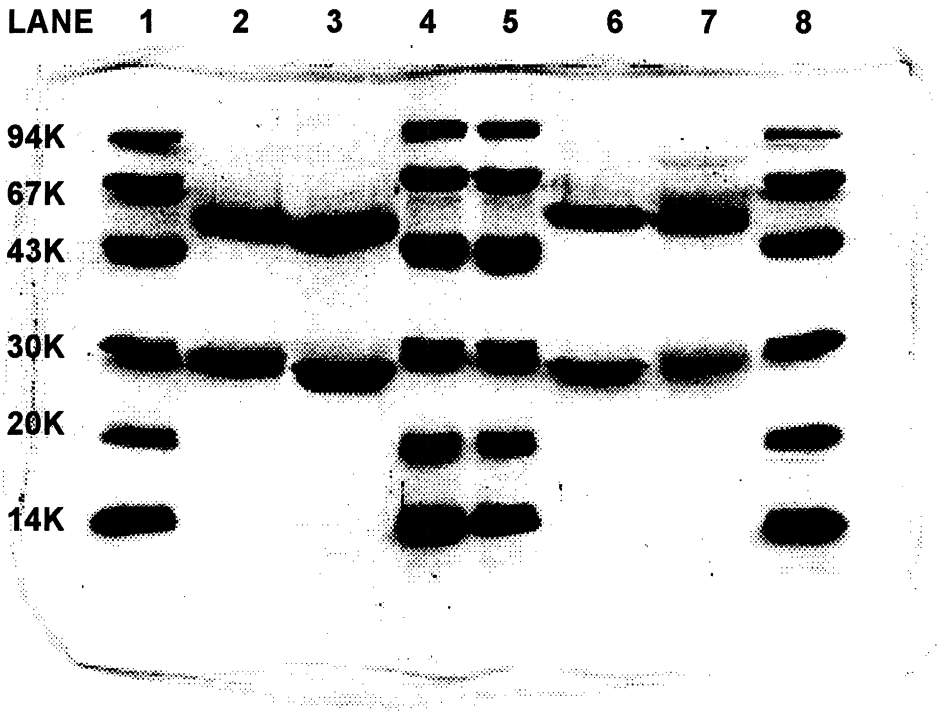
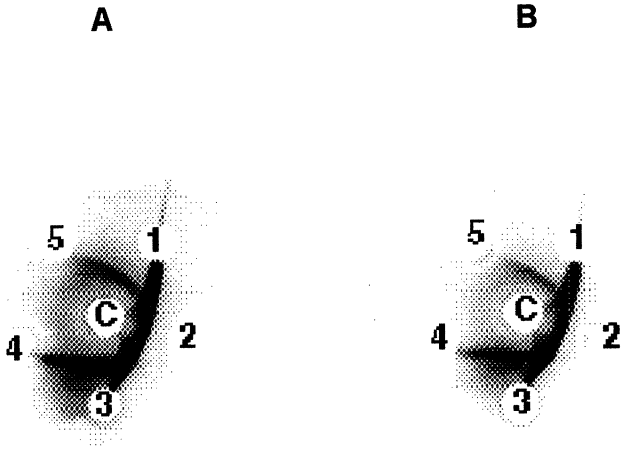

LANE 1 2 3 4 5 6 7 8
94K
67K
43K
30K
20K
14K

Figure 2

The Ouchterlony double diffusion method was used to identify the affinity purified monoclonal antibodies as rat IgG. Twenty-five μl of M1/70 (α-Mac-1; well 1), M3/38 (α-Mac-2; well 2), and B21-2 (α-I-$A^{b,d}$; well 3) was added to each well. Goat α-rat IgG (50 μl in the center well of A; 25 μl in the center well of B) was diffused against the monoclonal antibodies. The plate was incubated overnight at room temperature, and then stained with comassie blue. Precipitate bands were present at all wells containing M1/70, M3/38, and B21-2 monoclonal antibodies. Wells 4 and 5 were blank.


A
B
5
1
C
4
2
3
5
1
C
4
2
3

number (z-axis). In figure 3 a 3-D plot of Balb/c resident peritoneal macrophages labelled with normal rat IgG and rabbit α-rat IgG-FITC as a negative antibody control, exhibited background fluorescent intensity. Resident peritoneal macrophages labelled with α-Mac-1 and rabbit α-rat IgG-FITC exhibited high levels of fluorescence (figure 4), whereas resident peritoneal macrophages labelled with α-Mac-2 and rabbit α-rat IgG-FITC (figure 5) exhibited background fluorescent intensity similar to that seen in the negative control. Furthermore, resident peritoneal macrophages labelled with α-I-$A^{b,d}$ and rabbit α-rat IgG-FITC exhibited moderate levels of fluorescence (figure 6).

Peritoneal macrophages ellicited by an i.p. injection of thioglycollate and recovered six days postinoculation demonstrate a different pattern of Mac surface antigen expression. Thioglycollate-elicited peritoneal macrophages labelled with normal rat IgG and rabbit α-rat IgG-FITC exhibited background fluorescence (figure 7). In figure 8 thioglycollate-elicited peritoneal macrophages labelled with α-Mac-1 and rabbit α-rat IgG-FITC exhibited moderate levels of fluorescence; however, the thioglycollate-elicited peritoneal macrophages labelled with α-Mac-2 and rabbit α-rat IgG-FITC (figure 9) exhibited high fluorescent intensity, unlike resident peritoneal macrophages.

The immunofluorescence of resident peritoneal macrophages was determined by electronically gating on forward angle light scatter (size) and integrated 90° light scatter (granularity) signals. An electronic bitmap was drawn around the cell population with the size and granularity characteristics of macrophages, while carefully excluding granulocytes, lymphocytes, platelets, and red cells (figure 10). A typical resident peritoneal macrophage bitmap contains 35% of the forward angle light scatter and integrated 90°

Figure 3

Peritoneal cells recovered from control mice were labelled with normal rat IgG and rabbit α-rat IgG-FITC, and analyzed by flow cytometry. A 3-D plot of cell size (FALS) vs. cell fluorescence (IGFL) demonstrates the background level of fluorescence associated with control peritoneal cells, and the corresponding cell size distribution.

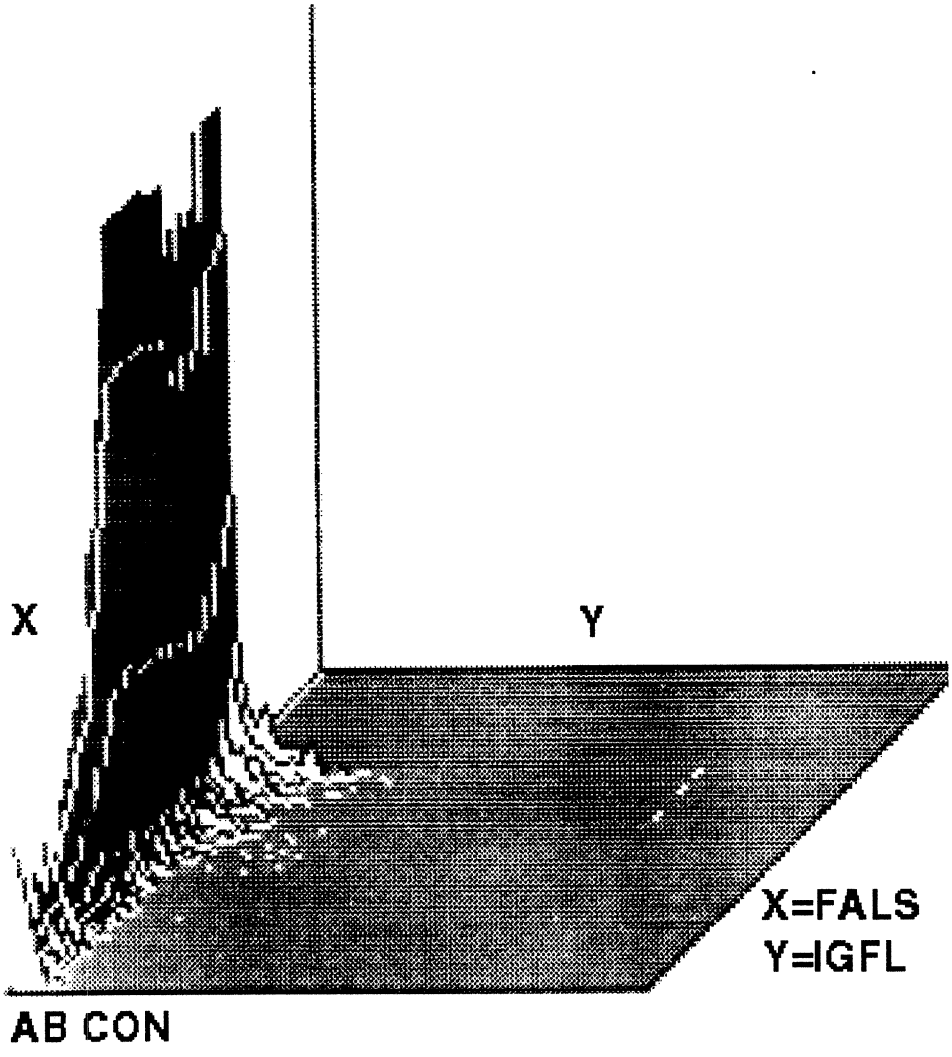

X
Y
X=FALS
Y=IGFL
AB CON

Figure 4

Peritoneal cells recovered from control mice were labelled with M1/70 (α-Mac-1) and rabbit α-rat IgG-FITC, and analyzed by flow cytometry. A 3-D plot of cell size (FALS) vs. cell fluorescence (IGFL) illustrates a shift to higher fluorescence for macrophage-sized cells, whereas lymphocyte-sized cells remain at background fluorescent levels.

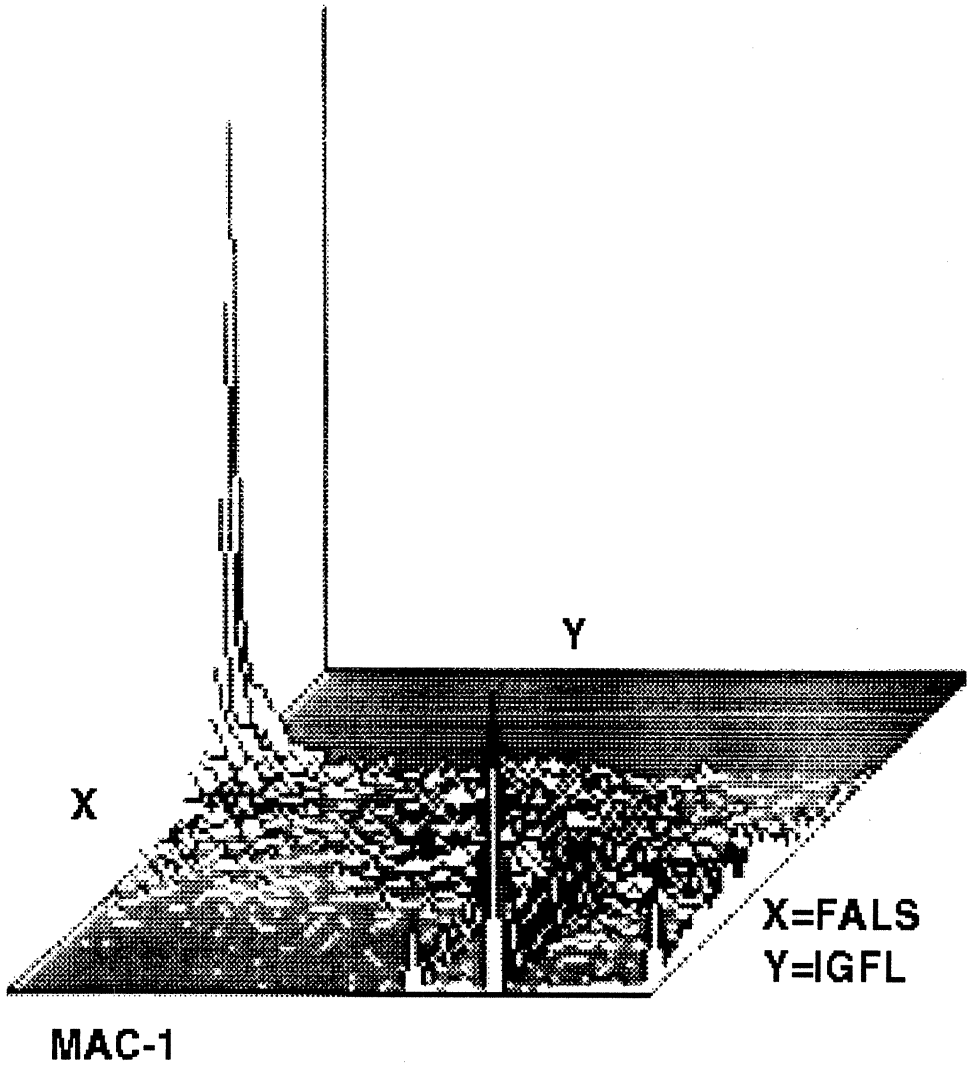

Y
X
X=FALS
Y=IGFL
MAC-1

Figure 5

Peritoneal cells recovered from control mice were labelled with M3/38 (α-Mac-2) and rabbit α-rat IgG-FITC, and analyzed by flow cytometry. A 3-D plot of cell size (FALS) vs. cell fluorescence (IGFL) demonstrates that a background level of fluorescence was associated with peritoneal cells labelled with M3/38.

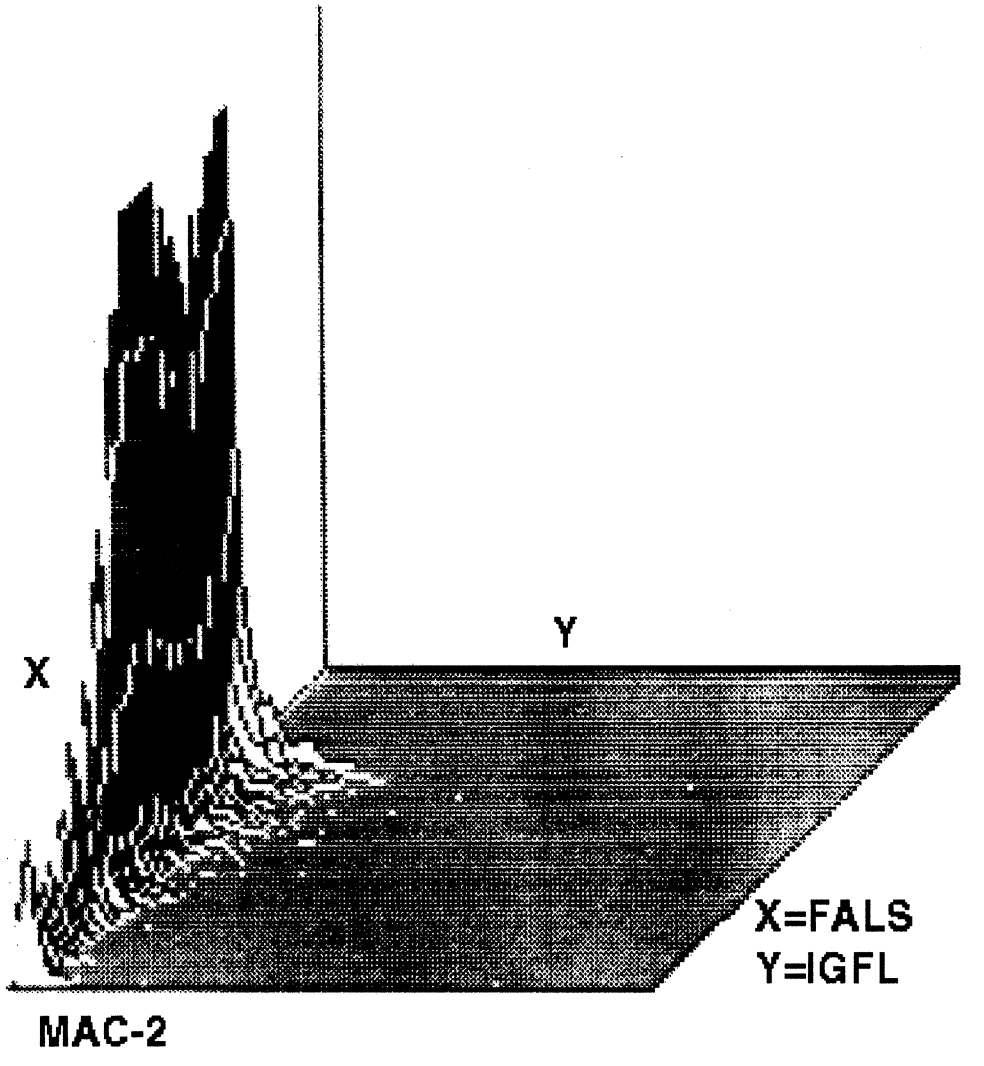

X
Y
X=FALS
Y=IGFL
MAC-2

Figure 6

Peritoneal cells recovered from control mice were labelled with B21-2 (α-I-$A^{b,d}$) and rabbit α-rat IgG-FITC, and analyzed by flow cytometry. A 3-D plot of cell size (FALS) vs. cell fluorescence (IGFL) illustrates that macrophage-sized cells express moderate levels of fluorescence, whereas lymphocyte-sized cells remain at background fluorescent levels.

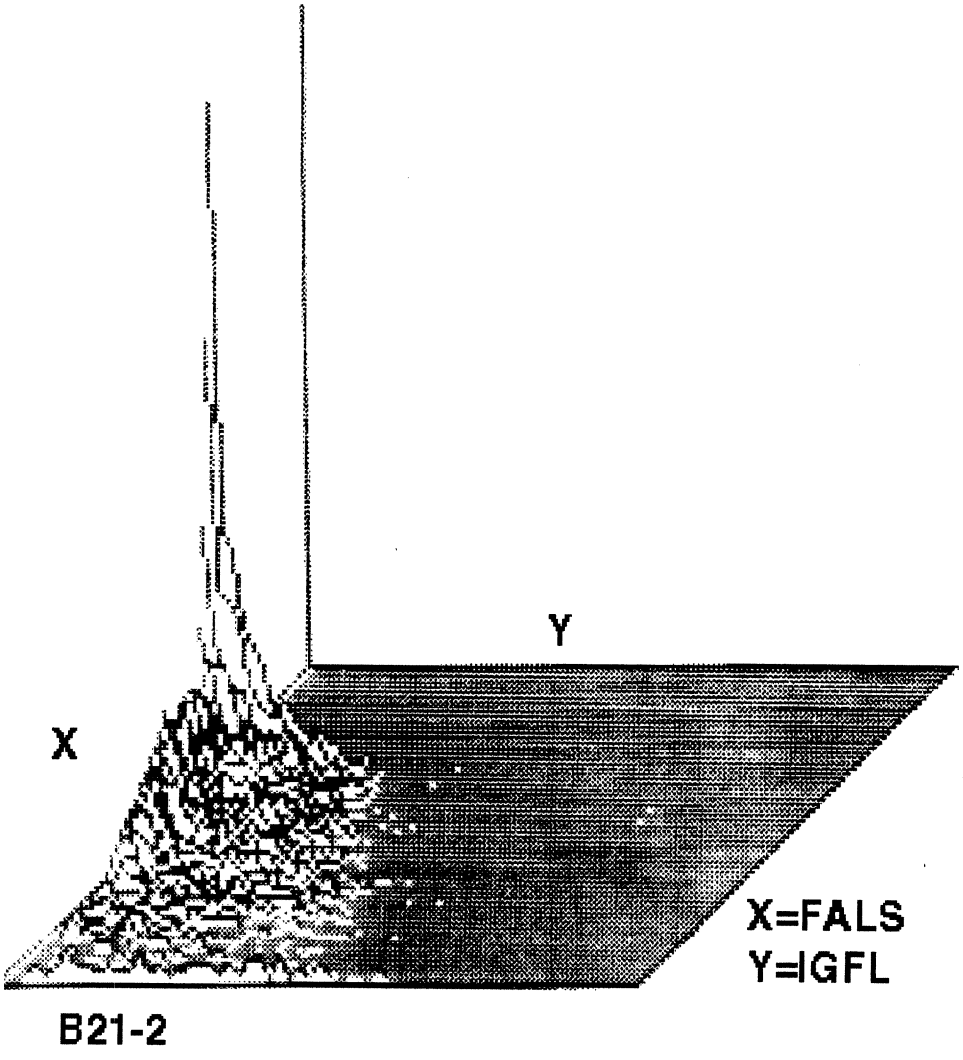

Y
X
X=FALS
Y=IGFL
B21-2

Figure 7

Peritoneal cells recovered from thioglycollate-primed mice (6 days PI) were labelled with normal rat IgG and rabbit α-rat IgG-FITC, and analyzed by flow cytometry. A 3-D plot of cell size (FALS) vs. cell fluorescence (IGFL) demonstrates the background level of fluorescence asscociated with thioglycollate-elicited peritoneal cells.

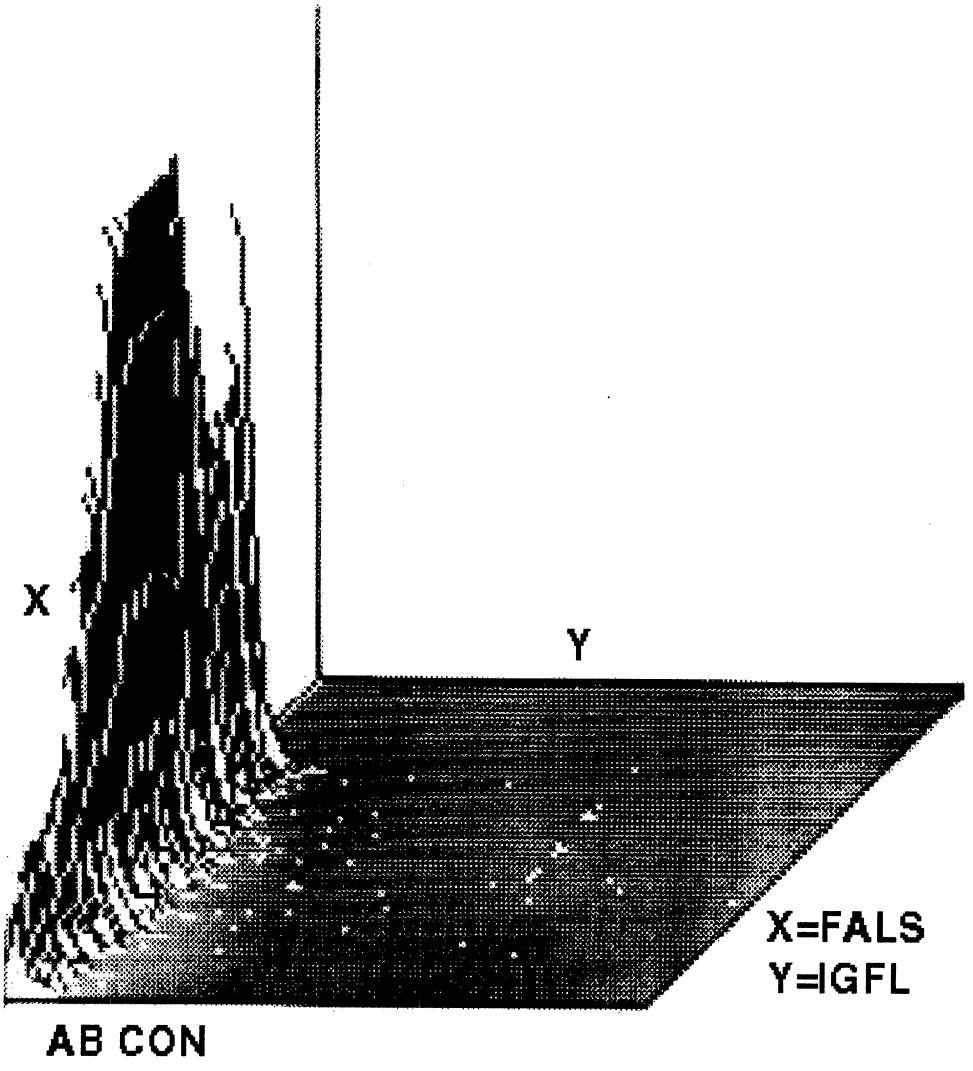

X
Y
X=FALS
Y=IGFL
AB CON

Figure 8

Peritoneal cells recovered from thioglycollate-primed mice (6 days PI) were labelled with M1/70 (α-Mac-1) and rabbit α-rat IgG-FITC, and analyzed by flow cytometry. A 3-D plot of cell size (FALS) vs. cell fluorescence (IGFL) illustrates that peritoneal macrophages elicited by thioglycollate express moderate levels of Mac-1 associated fluorescence.

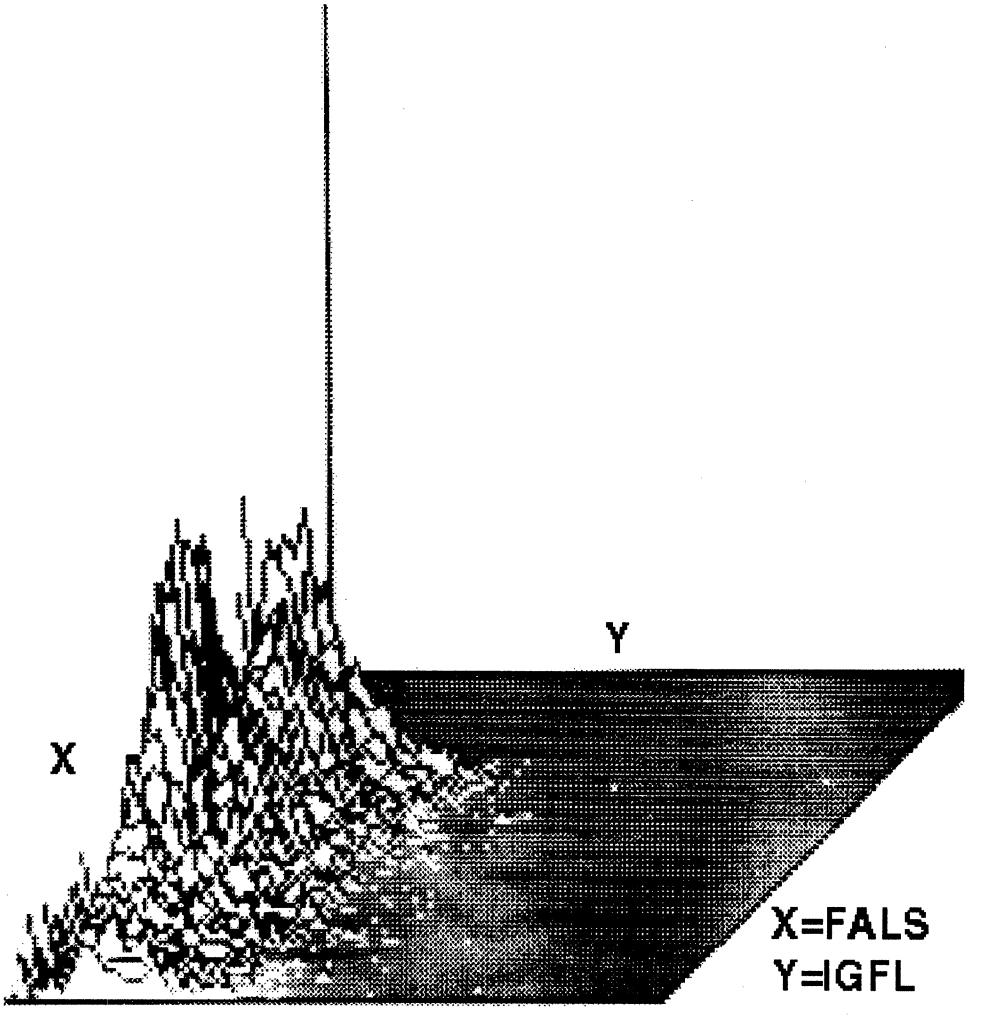

Y
X
X=FALS
Y=IGFL
MAC-1

Figure 9

Peritoneal cells recovered from thioglycollate-primed mice (6 days PI) were labelled with M3/38 (α-Mac-2) and rabbit α-rat IgG-FITC, and analyzed by flow cytometry. A 3-D plot of cell size (FALS) vs. cell fluorescence (IGFL) illustrates that thioglycollate-elicited peritoneal macrophages express high levels of Mac-2, whereas control macrophages do not express Mac-2 associated fluorescence.

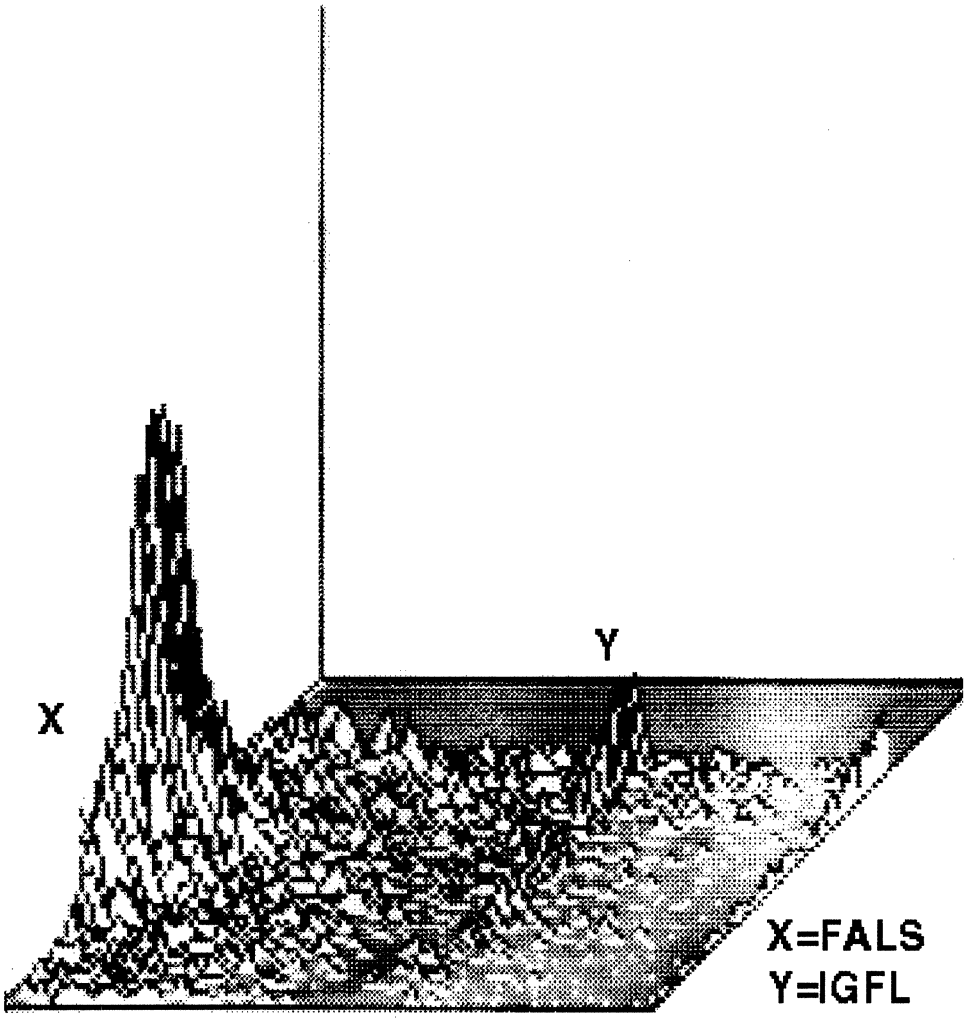
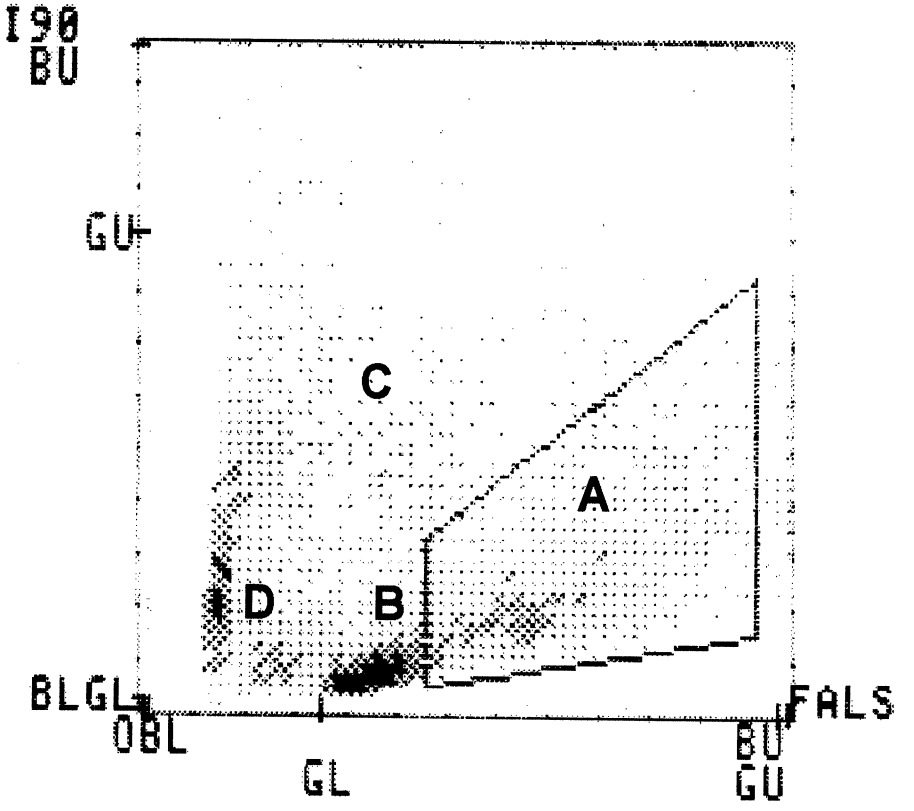

Y
X
X=FALS
Y=IGFL
MAC-2

Figure 10

Peritoneal cells recovered from control mice were analyzed by flow cytometry. A dual parameter dot plot of cell size (FALS) vs. cell granularity (integrated 90° light scatter [I90]) illustrates the representative populations of cells recovered from the peritoneum of mice. Region A is surrrounded by an electronic bitmap that allows the selection of signals originating from macrophage sized cells. The area plotted below B identifies lymphocytes; the area around C identifies granulocytes; the area around D identifies red cells (below), platelets (left), and cell debris (above).


I90
BU
GU
C
A
D
B
BLGL
OBL
GL
BU
GU
FALS

light scatter events. Log integrated green fluorescent histograms of resident peritoneal macrophages verses thioglycollate-ellicited peritoneal macrophages demonstrated the differential expression of Mac surface antigens (figure 11). The range of Mac-1 expression was similar in resident and thioglycollate-elicited macrophages, whereas the range of Mac-2 expression was higher in thioglycollate-elicited peritoneal macrophages. However, the Mac antigens and Ia antigen expression of resident peritoneal macrophages when compared to BCG cell wall-ellicited peritoneal macrophages was similar (figure 12).

Cellular Dynamics of Peritoneal Cells

Peritoneal cells were recovered from male Balb/c mice and counted during *T. spiralis* and *T. pseudospiralis* infections on days 4, 8, and 12 PI. Peritoneal cells were also recovered and counted from control and BCG cell wall-primed mice on day 6 PI. The *T. spiralis* and *T. pseudospiralis* infected mice demonstrated significantly higher peritoneal cell numbers on day 8 and 12 PI, than did control mice (table II). However, there were no significant differences between the *T. spiralis* and *T. pseudospiralis* values. Furthermore, BCG cell wall-primed mice exhibited significantly higher peritoneal cell numbers on day 6 PI, than did control mice (table II).

Peritoneal cell differential counts were done for *T. spiralis* and *T. pseudospiralis* infected mice on days 4, 8, and 12 PI. Also peritoneal cell differential counts were performed on control mice and BCG cell wall-primed mice on day 6 PI. The lymphocyte counts were significantly lower than control values during *T. spiralis* infections on day 8 PI, *T. pseudospiralis* infections on days 8 and 12 PI, and BCG cell wall-primed mice on day 6 PI (table III). Also there was a significant difference between *T. spiralis* and *T.*

Figure 11

Peritoneal cells recovered from control mice and thioglycollate-primed mice were incubated with normal rat IgG, M1/70 (α-Mac–1), or M3/38 (α-Mac-2). The cells were then labelled with rabbit α-rat IgG-FITC, and then analyzed by flow cytometry. Log integrated green fluorescence histograms comparing control peritoneal macrophages with thioglycollate-elicited peritoneal macrophages (6 days PI) illustrate that fluorescence associated with rat antibody controls or M1/70 labelled cells were similar. However, thioglycollate-elicited peritoneal macrophages demonstrate more Mac-2 associated fluorescence than control macrophages.

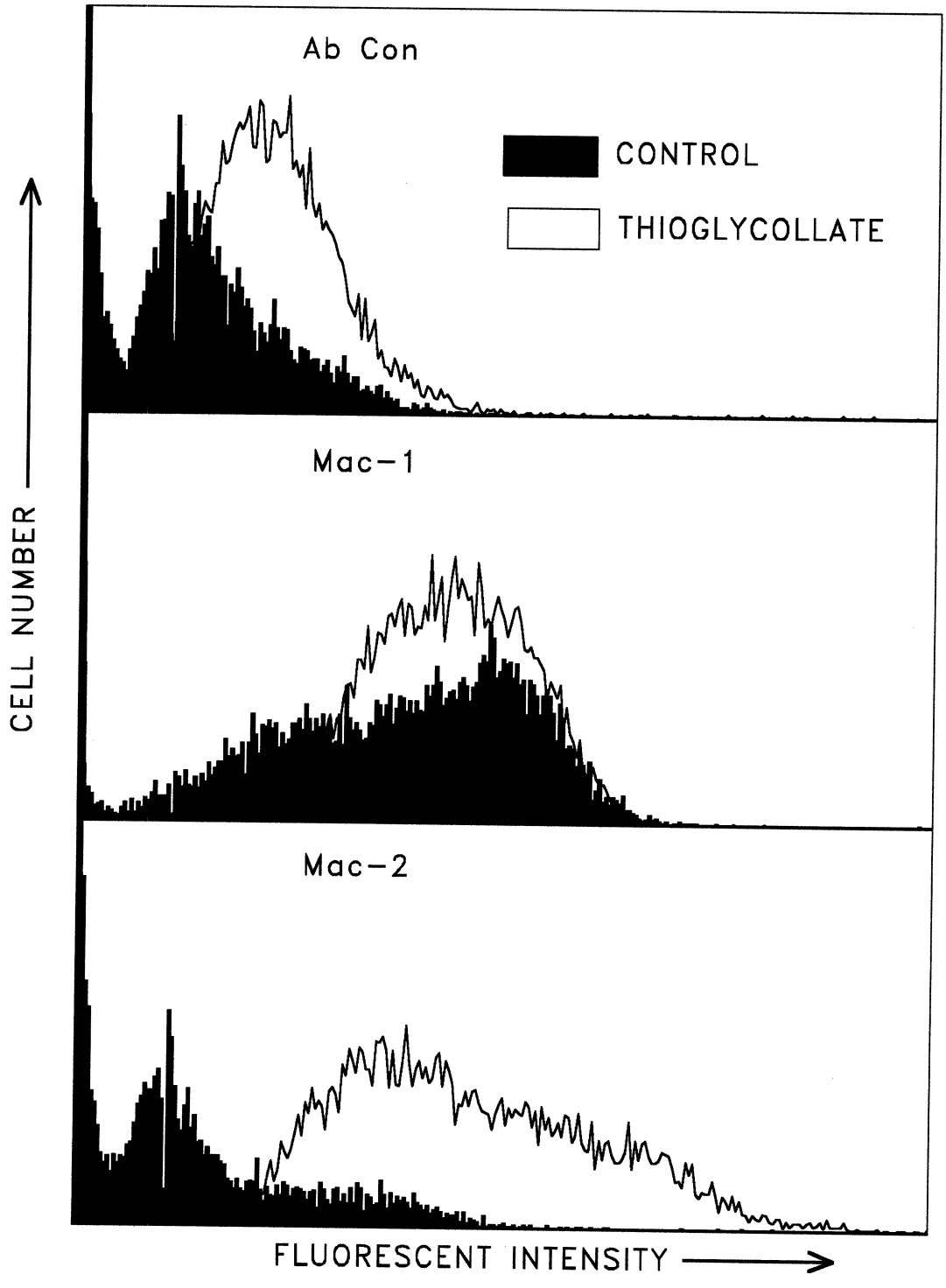

Ab Con
CONTROL
THIOGLYCOLLATE
Mac-1
Mac-2
CELL NUMBER
FLUORESCENT INTENSITY

Figure 12

Peritoneal cells recovered from control mice and BCG cell wall-primed mice (6 days PI) were incubated with normal rat IgG, M1/70 (α-Mac-1), M3/38 (α-Mac-2), or B21-2 (α-I-$A^{b,d}$). The cells were then labelled with rabbit α-rat IgG-FITC, and then analyzed by flow cytometry. LIGFL histograms comparing control peritoneal macrophages with BCG cell wall-elicited macrophages illustrate that fluorescence levels were similar for each of the antibodies tested.

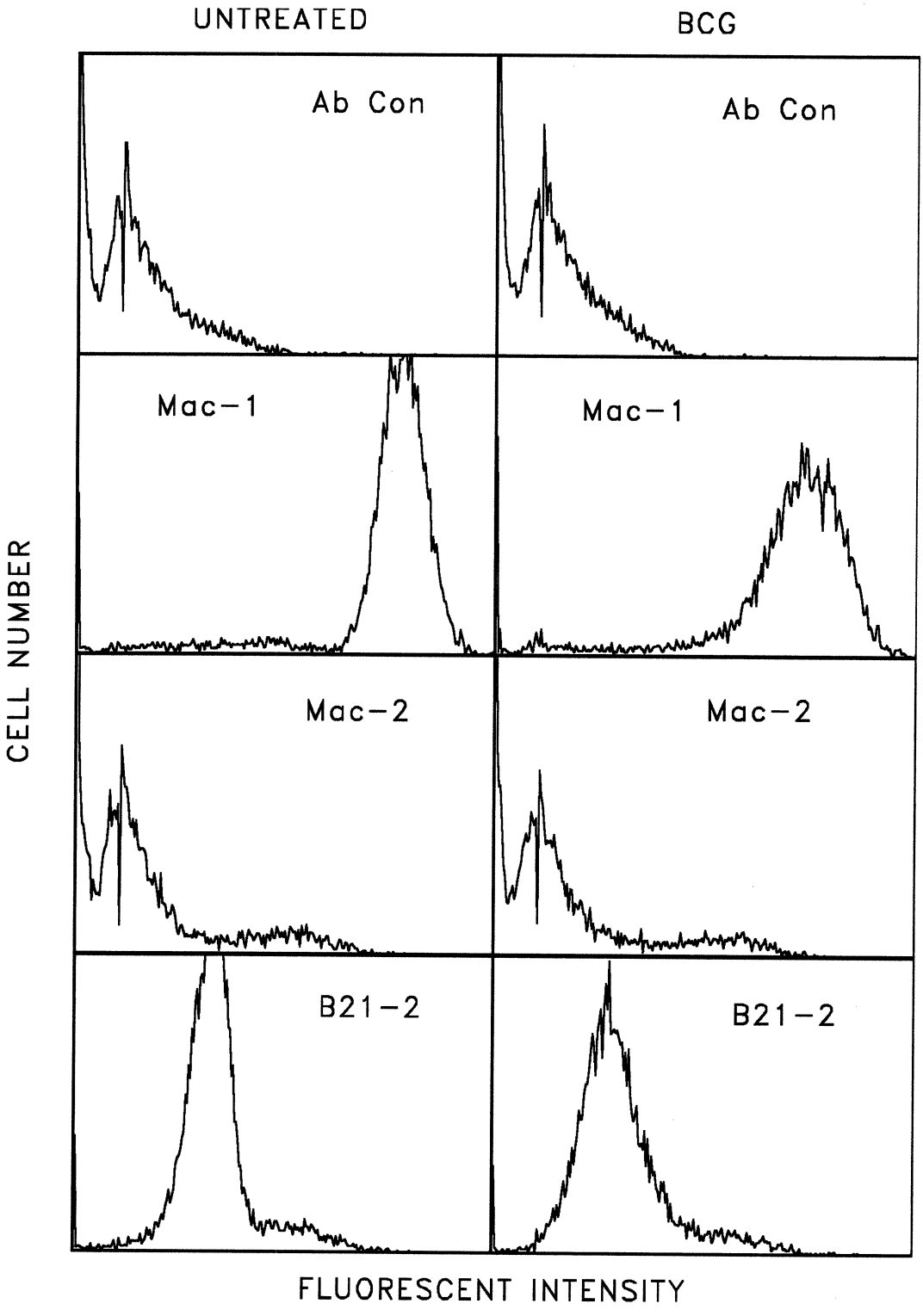

UNTREATED
BCG
Ab Con
Ab Con
Mac−1
Mac−1
Mac−2
Mac−2
B21−2
B21−2
CELL NUMBER
FLUORESCENT INTENSITY

Table II

Peritoneal cell number was determined for control, BCG cell wall primed, *T. spiralis* (500) infected, *T. pseudospiralis* (500) infected mice. Peritoneal cells were collected from *T. spiralis* or *T. pseudospiralis* infected mice on days 4, 8, and 12 PI, whereas cells from BCG cell wall primed mice were collected on day 6 PI. Values (mean ± SE) with a * beside them indicates that experimental values are significantly differrent than the control value ($P<0.05$).

Table II.

Peritoneal Cell Number ($x10^6$)

Day	*T. spiralis*	*T. pseudospiralis*	BCG Primed
Control	3.3 ± 0.1	3.6 ± 0.2	3.8 ± 0.2
4	3.3 ± 0.3	4.0 ± 0.3	—
6	—	—	9.6 ± 0.6*
8	12.8 ± 0.9*	11.6 ± 1.2*	—
12	16.6 ± 1.5*	16.7 ± 0.6*	—

* significantly different from control value ($P < 0.05$).

Table III

Peritoneal cell differrential counts were determined for control, BCG cell wall-primed, *T. spiralis* (500) infected, and *T. pseudospiralis* (500) infected mice. Peritoneal cells were collected from *T. spiralis* or *T. pseudospiralis*-infected mice on days 4, 8, and 12 PI, whereas cells were collected from BCG cell wall-primed mice on day 6 PI. Values (mean ± SE) with a † beside them indicates that the experimental value is significantly different from the control value. Likewise, values with a * beside them indicates a significant difference between *T. spiralis* and *T. pseudospiralis* values.

Table III.

Peritoneal Cell Differential Count

Day	Lymphocyte	Macrophage	Neutrophil	Eosinophil	Mast Cell
Control	43.0 ± 2.5	55.2 ± 2.5	0.7 ± 0.2	0.2 ± 0.2	1.0 ± 0.5
T. spiralis					
4	40.2 ± 5.4	56.3 ± 5.4	0.0± 0.0	1.5 ± 0.8	2.0 ± 0.4
8	30.3 ± 5.0†	68.7 ± 4.7†	0.2 ± 0.2	0.5 ± 0.2	0.3 ± 0.2
12	34.6 ± 3.1	56.8 ± 3.3	0.3 ± 0.2	7.8 ± 0.8†	0.3 ± 0.2
T. pseudospiralis					
4	44.2 ± 1.9	53.0 ± 2.0	0.3 ± 0.2	1.2 ± 0.5	1.3 ± 0.6
8	24.5 ± 1.8†	74.2 ± 1.7†	0.2 ± 0.2	0.8 ± 0.5	0.3 ± 0.2
12	28.5 ± 1.5†	65.5 ± 1.7*†	0.0 ± 0.0	5.7 ± 0.8†	0.5 ± 0.3
BCG Primed					
6	25.3 ± 2.6†	67.8 ± 2.6†	1.2 ± 0.7	5.5 ± 1.2†	0.2 ± 0.2

* significant difference between *T. spiralis* and *T. pseudospiralis* values ($P < 0.05$).
† significantly different from control value ($P < 0.05$).

pseudospiralis groups, with the macrophage percentage of the *T. pseudospiralis* group higher than the *T. spiralis* group on day 12 PI (table III). Additionally, the eosinophil counts were significantly higher than control values during *T. spiralis* infections on day 12 PI, *T. pseudospiralis* infections on day 12 PI, and BCG cell wall primed mice on day 6 PI (table III). There were no significant differences observed in neutrophil or mast cell counts.

Peritoneal Macrophage Subpopulation Numbers

Absolute macrophage numbers were calculated by multiplying the total peritoneal cell number by the decimal proportion representing peritoneal macrophages (differential count). Likewise, the absolute Mac-1, Mac-2, and Ia positive cell numbers were calculated by multiplying the total peritoneal cell number by the decimal proportion of Mac-1, Mac-2, and Ia positive macrophages as determined by flow cytometry. The *T. spiralis* and *T. pseudospiralis*-infected mice demonstrated significantly higher absolute macrophage numbers, Mac-1 positive cell numbers, and Ia positive cell numbers on days 8 and 12 PI, than did the control mice (table IV). On the other hand, *T. spiralis* and *T. pseudospiralis*-infected mice exhibited absolute Mac-2 positive cell numbers that were significantly higher than the control values only on day 12 PI (table IV). A significant difference between the *T. spiralis* and *T. pseudospiralis* groups was detected for absolute macrophage numbers on day 12 PI, with the *T. pseuodospiralis* value higher than the *T. spiralis* value (table IV). The BCG cell wall primed mice demonstrated significantly higher values for absolute macrophage, Mac-1 positive, Mac-2 positive, and Ia positive cell numbers, than did the control mice (table IV).

Table IV

Peritoneal macrophage subpopulation numbers were determined for control, BCG cell wall-primed, *T. spiralis* (500) infected, and *T. pseudospiralis* (500) infected mice. Peritoneal cells were collected from *T. spiralis* or *T. pseudospiralis*-infected mice on days 4, 8, and 12 PI, whereas cells were collected from BCG cell wall-primed mice on day 6 PI. The cells were then analyzed by flow cytometry to determine surface marker expression, and total and differential cell counts were performed. The absolute macrophage number was determined by multiplying the decimal percentage of macrophages by the total cell number. The absolute Mac-1, Mac-2, and Ia expressing populations were determined by multiplying the decimal percentage of surface marker expression (flow cytometry) by the total cell number. Values (mean ± SE) with a † beside them indicate that the experimental value is significantly different from the control value. Likewise, values with a * beside them indicate a significant difference between *T. spiralis* and *T. pseudospiralis* values.

Table IV.

Peritoneal Macrophage Subpopulation Numbers ($x10^6$)

Day	Percent MØ	Absolute MØ	Absolute Mac-1	Absolute Mac-2	Absolute Ia
Control	55.2	2.0 ± 0.1	1.4 ± 0.1	0.09 ± 0.01	1.4 ± 0.1
T. spiralis					
4	56.4	1.9 ± 0.2	1.3 ± 0.1	0.12 ± 0.03	1.4 ± 0.1
8	68.7	8.8 ± 0.6†	7.3 ± 0.7†	0.39 ± 0.2	6.9 ± 0.4†
12	56.9	9.4 ± 0.5†	8.2 ± 0.8†	0.56 ± 0.2†	7.8 ± 0.6†
T. pseudospiralis					
4	53.0	2.1 ± 0.1	1.5 ± 0.1	0.07 ± 0.01	1.6 ± 0.1
8	74.2	8.6 ± 0.2†	6.7 ± 0.1†	0.36 ± 0.13	6.6 ± 0.1†
12	65.5	10.9 ± 0.3*†	9.2 ± 0.2†	0.79 ± 0.02†	8.8 ± 0.2†
BCG Primed					
6	67.8	5.6 ± 0.2†	4.7 ± 0.2†	0.45 ± 0.02†	3.9 ± 0.1†

* significant difference between *T. spiralis* and *T. pseudospiralis* values ($P < 0.05$).
† significantly different from control value ($P < 0.05$).

LPS Stimulated Prostaglandin E Production

Peritoneal macrophages were recovered from control and BCG cell wall-primed mice and were incubated with different concentrations of LPS. The production of PGE by cultured peritoneal macrophages was determined by radioimmunoassay. Peritoneal macrophages from untreated control mice responded to LPS stimulation in a dose dependent manner, with as little as 1 μg of LPS stimulating PGE production. In the untreated control peritoneal macrophage cultures, LPS stimulated PGE production peaked and maintained a plateau at doses of 10, 25, and 50 μg of LPS (figure 13). However, BCG cell wall-primed peritoneal macrophage cultures demonstrated 2-3 fold increases in PGE production after LPS stimulation. The PGE levels in culture supernatants from BCG cell wall-primed macrophages were significantly higher than those from control cultures at the 5, 10, 25, and 50 μg concentrations of LPS (figure 13).

Peritoneal macrophages recovered from control mice and mice infected with *T. spiralis* or *T. pseudospiralis* on days 4, 8, and 12 PI were incubated with or without 25 μg of LPS. There were no significant differences between *T. spiralis* and *T. pseudospiralis* groups in either untreated or LPS treated cultures. Likewise, there was no significant difference between controls and experimental PGE production. However, in the LPS treated cultures the PGE levels peaked at day 4 PI and declined through day 12 PI, with *T. pseudospiralis* levels slightly higher than *T. spiralis* PGE levels (figure 14). The trend in PGE levels for untreated cultures was increasing PGE levels during the *T. spiralis* infection, whereas *T. pseudospiralis* PGE levels decreased slightly during the sequence (figure 14).

Figure 13

Peritoneal cells were collected from control and BCG cell wall-primed mice (6 days PI) were cultured with 1, 5, 10, 25, and 50 μg/ml concentrations of LPS. After 24 hours the culture supernatants were harvested and the PGE was extracted. The quantity of PGE was determined by radioimmunoassay. BCG symbols with a * (above) are significantly greater than LPS values ($P<0.05$).

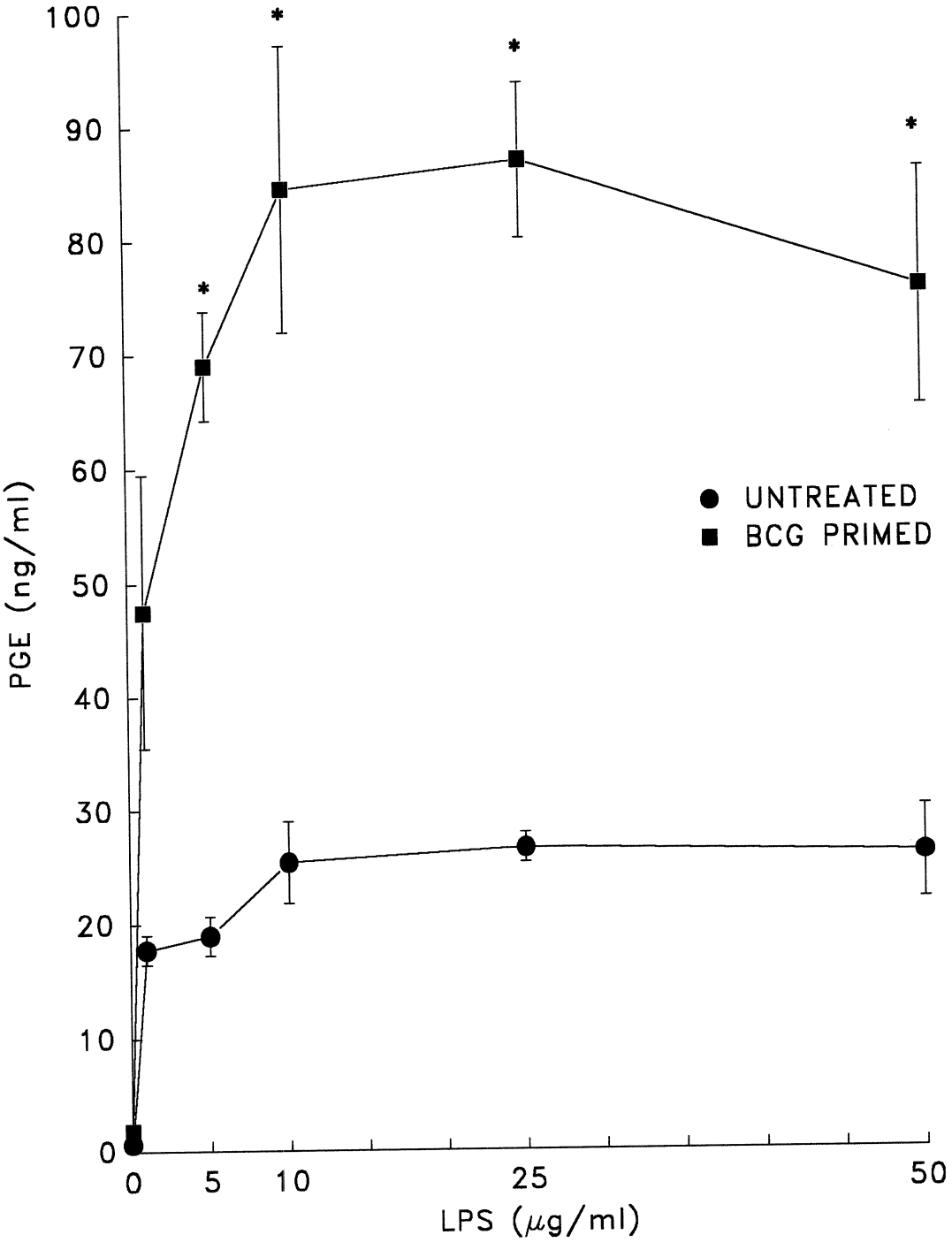
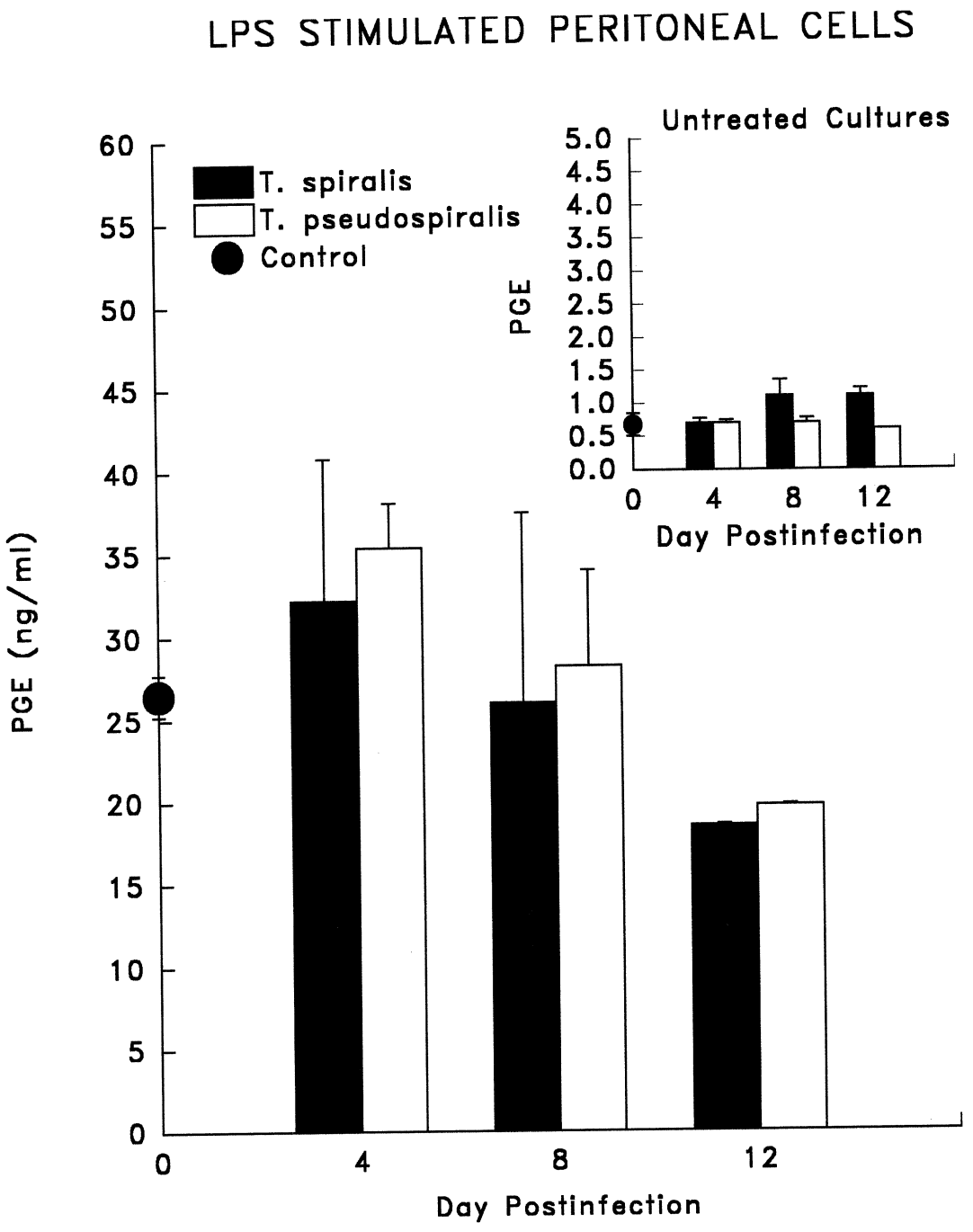

100
90
80
70
60
50
40
30
20
10
0
PGE (ng/ml)
*
*
*
*
UNTREATED
BCG PRIMED
0
5
10
25
50
LPS (μg/ml)

Figure 14

Peritoneal cells collected from control, *T. spiralis* (500) infected , or *T. pseudospiralis* (500) infected mice were cultured without LPS (untreated cultures), or with 25 μg/ml of LPS. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. Bar heights are the mean ± SE (on top).


LPS STIMULATED PERITONEAL CELLS
T. spiralis
T. pseudospiralis
Control
PGE (ng/ml)
60
55
50
45
40
35
30
25
20
15
10
5
0
0
4
8
12
Day Postinfection
Untreated Cultures
PGE
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
4
8
12
Day Postinfection

PGE Levels in Mouse Peritoneal Fluid

Mouse peritoneal lavage fluid recovered from mice infected with *T. spiralis* or *T. pseudospiralis* was assayed to determine PGE levels. PGE levels were assayed on days 4, 8, and 12 PI, along with control lavage fluid. The PGE level for *T. pseudospiralis* day 4 PI was remarkably higher than the *T. spiralis* value; however, the difference was not significant (figure 15). The PGE levels for control, day 8 PI, and day 12 PI were similar (figure 15).

PGE Production Induced by *Trichinella* Soluble Antigens

Peritoneal macrophages were recovered from control (unprimed) and BCG cell wall-primed mice prior to incubation with different doses of *T. spiralis* or *T. pseudospiralis* antigen extracts. Antigen extracts from the three major lifecycle stages of *Trichinella* were used: The adult (reproductive stage), new born larvae (migratory stage), and muscle larvae (infective stage). Figure 16 shows the result of PGE radioimmunoassays of *T. spiralis* antigen extract stimulated macrophage culture supernatants. *T. spiralis* newborn larvae antigen extract elicited the greatest PGE response at 1, 5, and 10 μg doses, whereas the *T. spiralis* adult antigen extract at 10, 25, and 50 μg doses elicited PGE responses that were lower than the *T. spiralis* newborn larvae antigen extract. However, *T. spiralis* adult antigen elicited a higher PGE response than *T. spiralis* muscle larvae antigen at the same dose levels (figure 16). Figure 17 shows PGE levels from unprimed macrophage cultures after stimulation with various doses of *T. pseudospiralis* antigen extracts. The *T. pseudospiralis* newborn larvae antigen demonstrated the highest potency for stimulating PGE production. Similarly, *T. pseudospiralis* adult antigen stimulated high PGE levels, yet

Figure 15

Peritoneal lavage fluid collected from control, *T. spiralis* (500) infected, or *T. pseudospiralis* (500) infected mice was processed and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. Bar heights are the mean ± SE (on top).

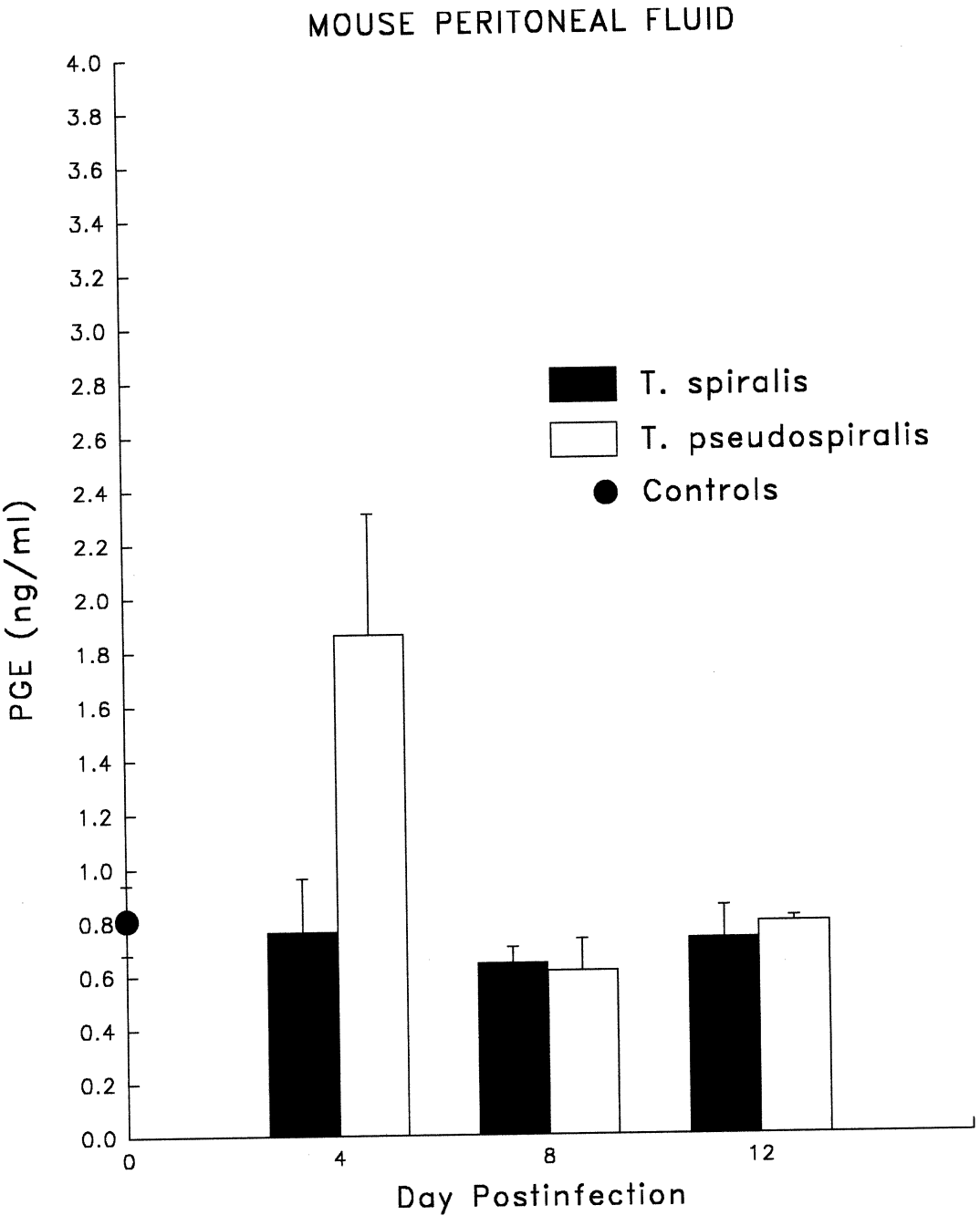

MOUSE PERITONEAL FLUID
PGE (ng/ml)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
4
8
12
Day Postinfection
T. spiralis
T. pseudospiralis
Controls

Figure 16

Peritoneal cells collected from unprimed mice were cultured with 0, 10, 25, and 50 μg/ml concentrations of *T. spiralis* adult or muscle larvae antigens. Also cells were cultured with 0, 1, 5, and 10 μg/ml concentrations of *T. spiralis* newborn larvae antigen. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE.

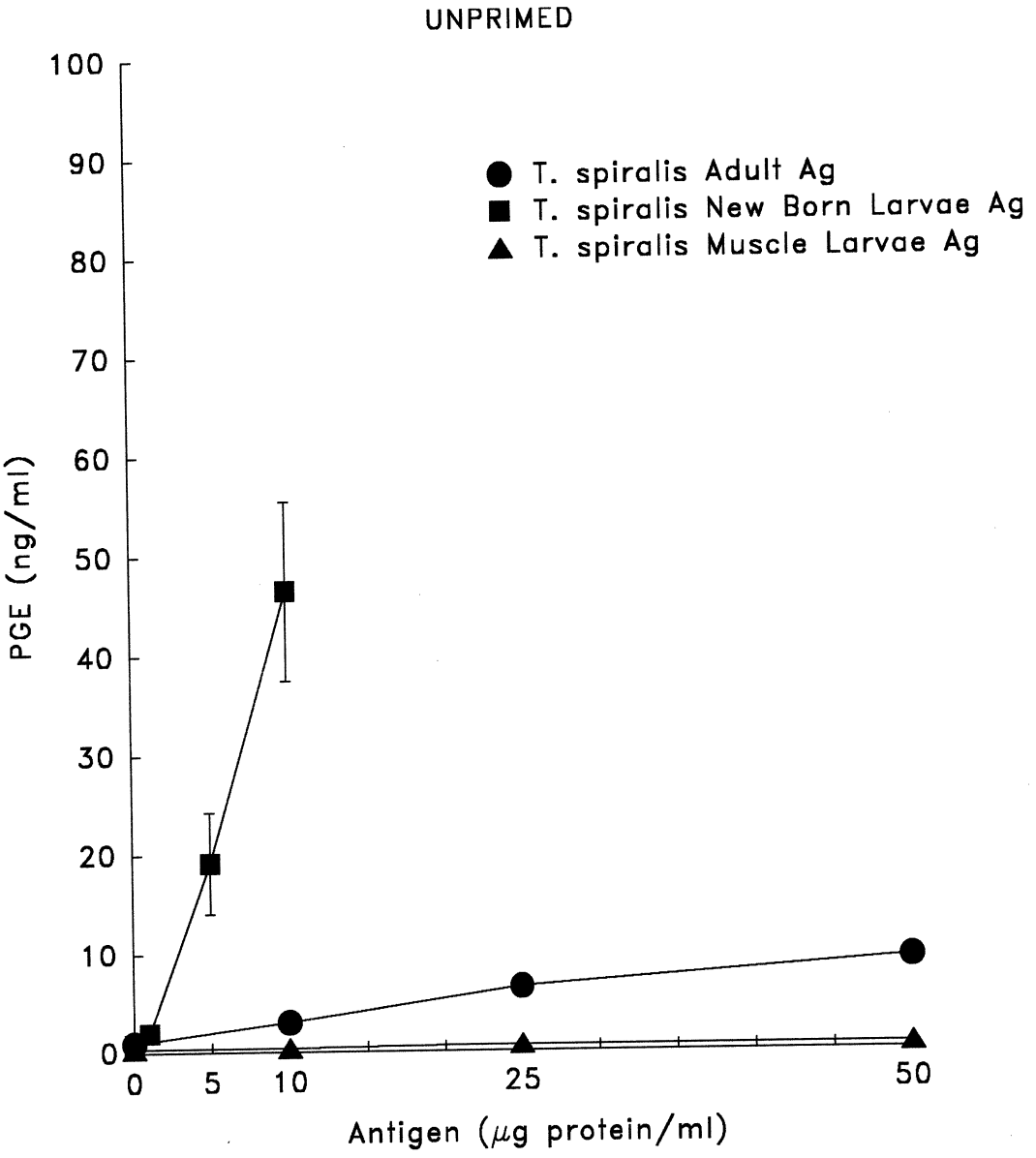
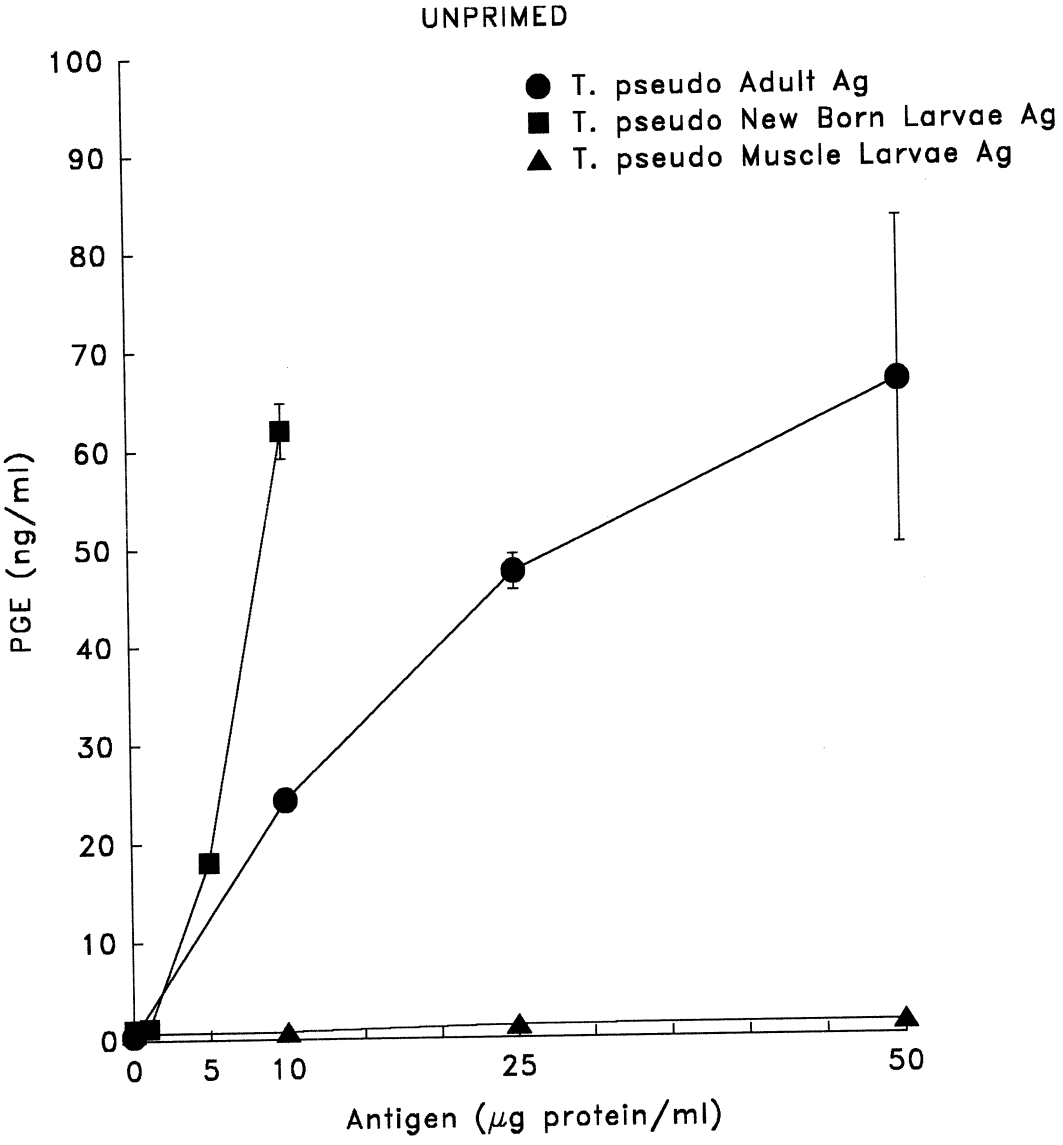

UNPRIMED
T. spiralis Adult Ag
T. spiralis New Born Larvae Ag
T. spiralis Muscle Larvae Ag
PGE (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
25
50
Antigen (μg protein/ml)

Figure 17

Peritoneal cells collected from unprimed mice were cultured with 0, 10, 25, and 50 μg/ml concentrations of *T. pseudospiralis* adult or muscle larvae antigens. Also cells were cultured with 0, 1, 5, and 10 μg/ml concentrations of *T. pseudospiralis* newborn larvae antigen. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE.


UNPRIMED
T. pseudo Adult Ag
T. pseudo New Born Larvae Ag
T. pseudo Muscle Larvae Ag
PGE (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
25
50
Antigen (μg protein/ml)

at higher concentrations of antigen extract than *T. pseudospiralis* newborn larvae extracts. Furthermore, *T. pseudospiralis* muscle larvae antigen elicited the lowest PGE response by macrophages (figure 17).

Peritoneal macrophages recovered from BCG cell wall-primed mice demonstrated an enhanced capability to produce PGE after stimulation with *Trichinella* antigens. Figure 18 shows that the pattern of response to *T. spiralis* newborn larvae, adult, and muscle larvae antigens was similar to those seen in the unprimed macrophage cultures. However, the PGE response was higher at almost all antigen concentrations regardless of which *T. spiralis* stage antigen extract was used (figure 18). Likewise, macrophages from BCG cell wall-primed mice stimulated with *T. pseudospiralis* newborn larvae, adult, or muscle larvae antigen extract, produce more PGE than *T. pseudospiralis* antigen stimulated macrophages from unprimed mice (figure 19). The greatest difference was exhibited by macrophages stimulated with *T. pseudospiralis* adult antigen, where the peak PGE production occurred at the 25 μg dose of adult antigen, when compared to the 50 μg dose response of unprimed peritoneal macrophages (figure 19).

When PGE production by peritoneal macrophages stimulated by *T. spiralis* or *T. pseudospiralis* antigens are compared some remarkable differences appear. PGE levels produced by *T. pseudospiralis* adult antigen stimulated macrophages, from unprimed mice, were significantly higher at the 10, 25, and 50 μg concentrations, than the PGE levels produced by unprimed macrophages stimulated with *T. spiralis* adult antigen at the same concentrations (figure 20). On the other hand, there was no significant difference between the PGE response of macrophages from unprimed mice that were stimulated

Figure 18

Peritoneal cells collected from BCG cell wall-primed mice were cultured with 0, 10, 25, and 50 μg/ml concentrations of *T. spiralis* adult or muscle larvae antigens. Also cells were cultured with 0, 1, 5, and 10 μg/ml concentrations of *T. spiralis* newborn larvae antigen. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE.

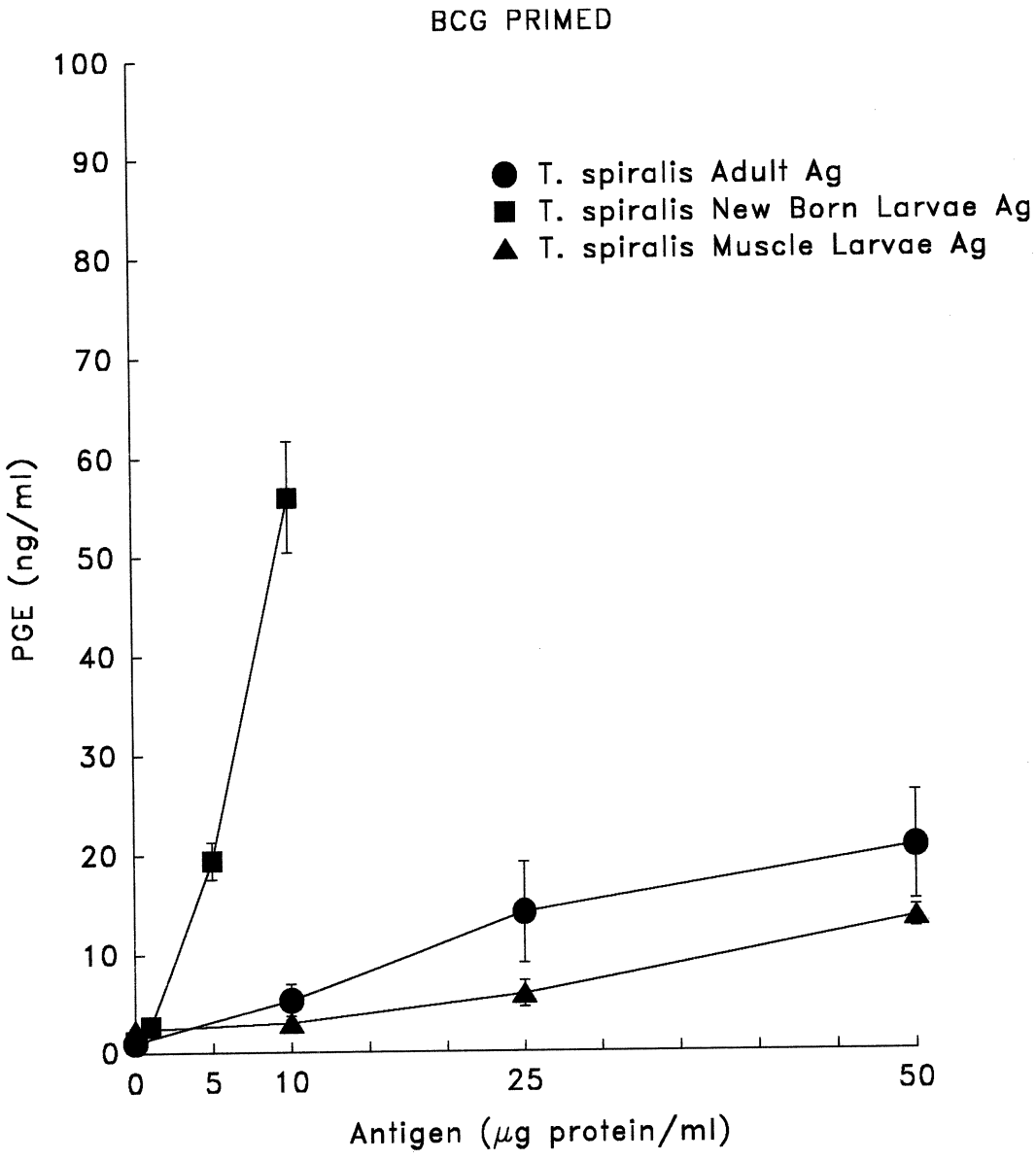

BCG PRIMED
T. spiralis Adult Ag
T. spiralis New Born Larvae Ag
T. spiralis Muscle Larvae Ag
PGE (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
25
50
Antigen (μg protein/ml)

Figure 19

Peritoneal cells collected from BCG cell wall-primed mice were cultured with 0, 10, 25, and 50 μg/ml concentrations of *T. pseudospiralis* adult or muscle larvae antigens. Also cells were cultured with 0, 1, 5, and 10 μg/ml concentrations of *T. pseudospiralis* newborn larvae antigen. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE.

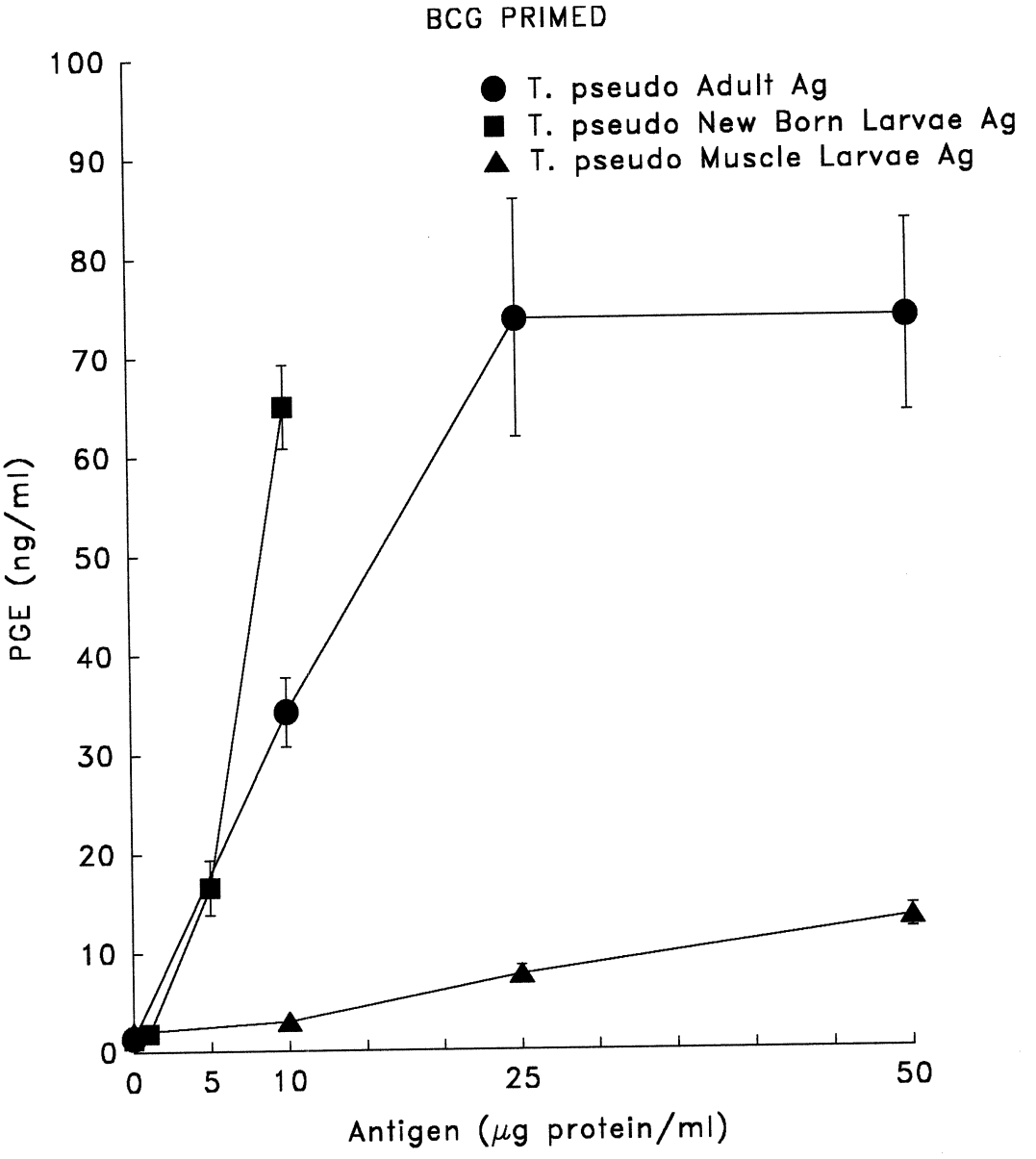
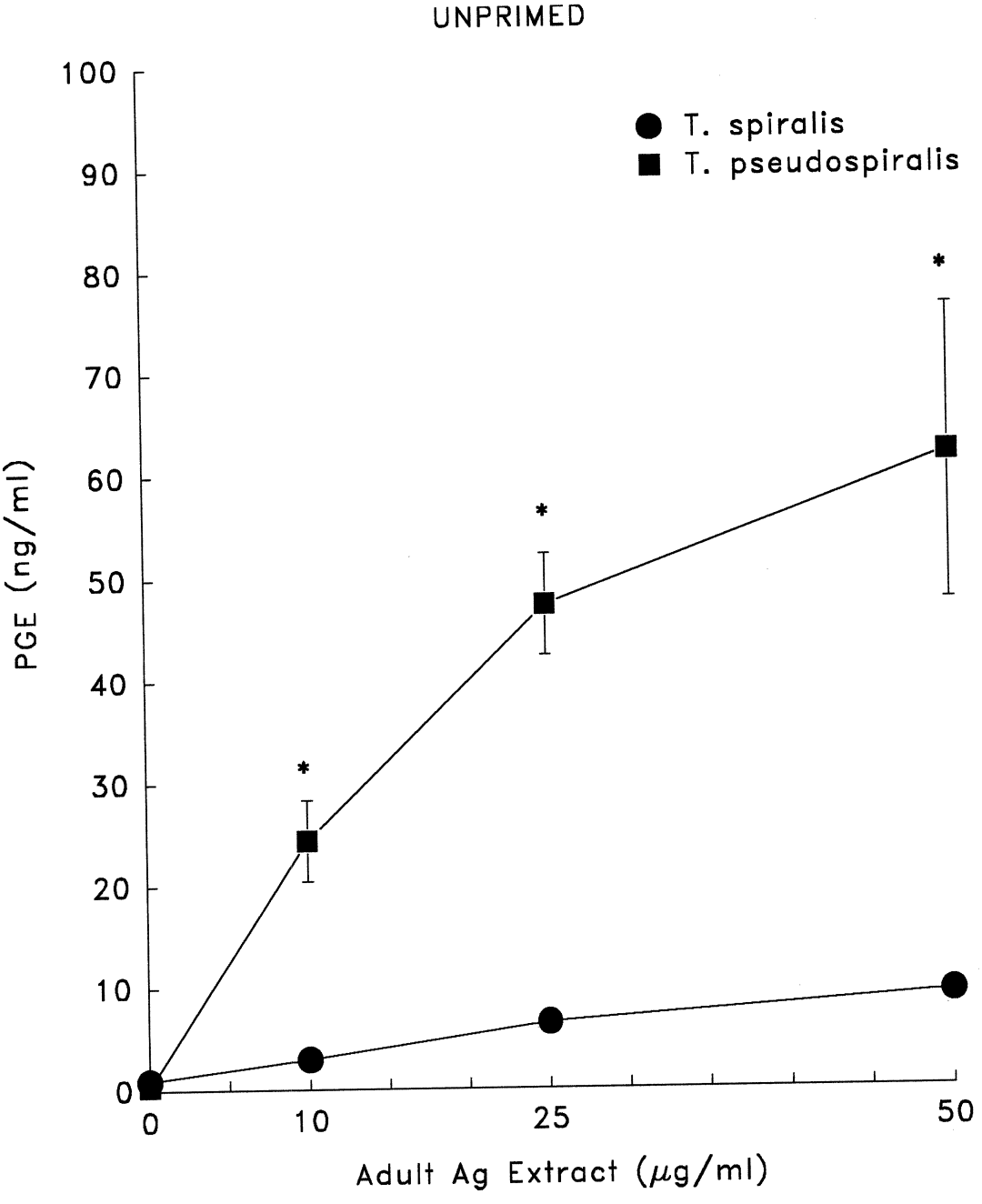

BCG PRIMED
T. pseudo Adult Ag
T. pseudo New Born Larvae Ag
T. pseudo Muscle Larvae Ag
PGE (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
25
50
Antigen (μg protein/ml)

Figure 20

Peritoneal cells collected from unprimed mice were cultured with 0, 10, 25, and 50 μg/ml concentrations of *T. spiralis* or *T. pseudospiralis* adult antigens. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE of at least two experiments. A * above the symbol indicates a significant difference between *T. spiralis* and *T. pseudospiralis* values ($P<0.05$).


UNPRIMED
T. spiralis
T. pseudospiralis
PGE (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
*
*
*
0
10
25
50
Adult Ag Extract (μg/ml)

with *T. spiralis* or *T. pseudospiralis* newborn larvae antigen extract (figure 21). However *T. pseudospiralis* muscle larvae antigen elicited a significantly higher PGE response by macrophages from unprimed mice at doses of 10 and 25 μg, than did macrophages from unprimed mice stimulated with *T. spiralis* muscle larvae antigen at the same doses (figure 22).

Macrophages from BCG cell wall-primed mice provided a another comparison of *T. spiralis* and *T. pseudospiralis* antigen potency. *T. pseudospiralis* adult antigen stimulated macrophages from BCG cell wall primed mice demonstrated significantly higher PGE levels at 10, 25, and 50 μg doses, than the PGE levels expressed by macrophages recovered from BCG cell wall-primed mice after stimulation with *T. spiralis* adult antigen at the same doses (figure 23). However, there were no significant differences between *T. spiralis* and *T. pseudospiralis* newborn larvae antigen stimulated macrophages from BCG cell wall-primed mice (figure 24). Likewise, there was little difference between the levels of PGE expressed by *T. spiralis* and *T. pseudospiralis* muscle larvae antigen stimulated macrophages from BCG cell wall-primed mice (figure 25).

Figure 21

Peritoneal cells collected from unprimed mice were cultured with 0, 1, 5, and 10 μg/ml concentrations of *T. spiralis* or *T. pseudospiralis* newborn larvae antigens. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE of at least two experiments.

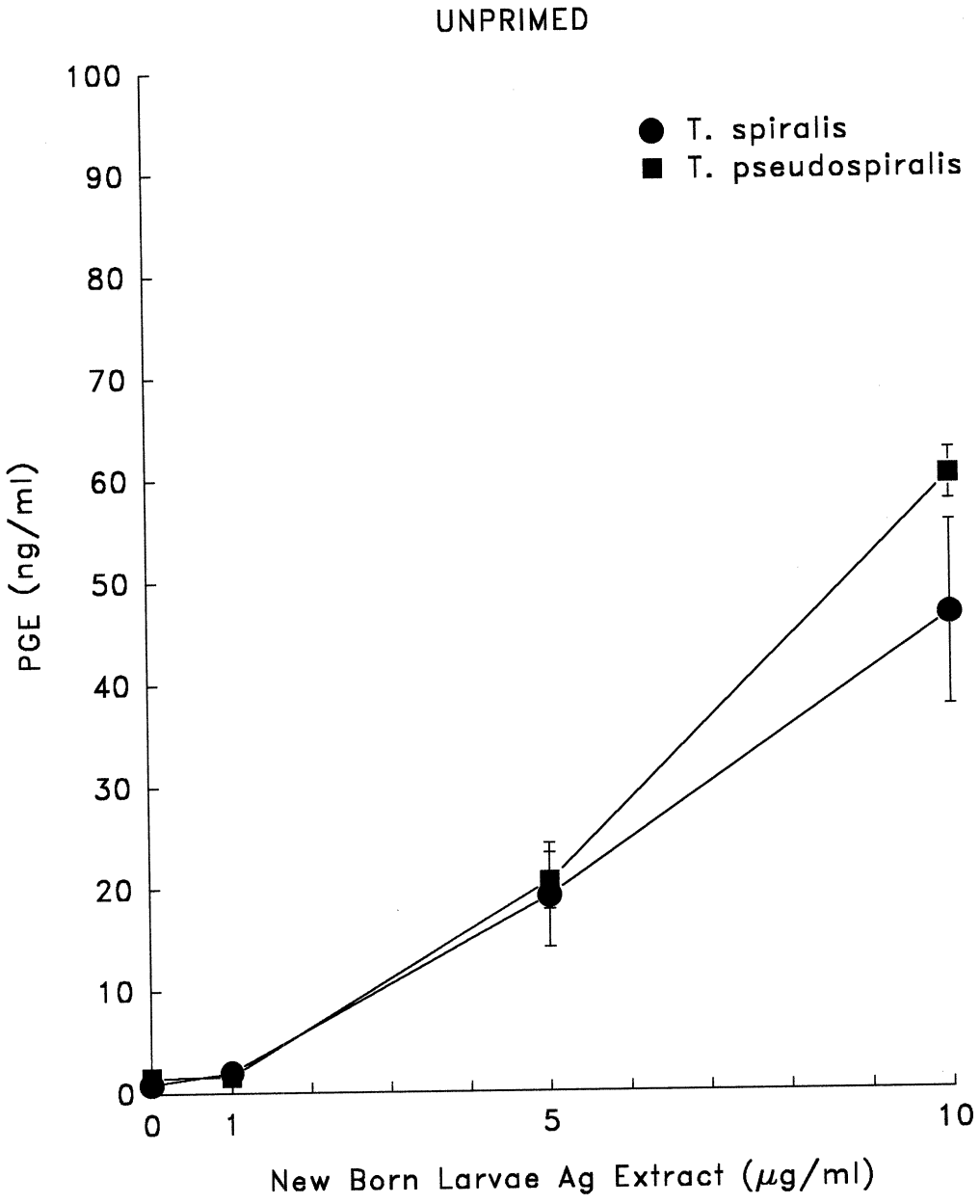

UNPRIMED
T. spiralis
T. pseudospiralis
PGE (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
0
1
5
10
New Born Larvae Ag Extract (μg/ml)

Figure 22

Peritoneal cells collected from unprimed mice were cultured with 0, 10, 25, and 50 μg/ml concentrations of *T. spiralis* or *T. pseudospiralis* muscle larvae antigens. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE. A * above the symbol indicates a significant difference between *T. spiralis* and *T. pseudospiralis* values ($P<0.05$).

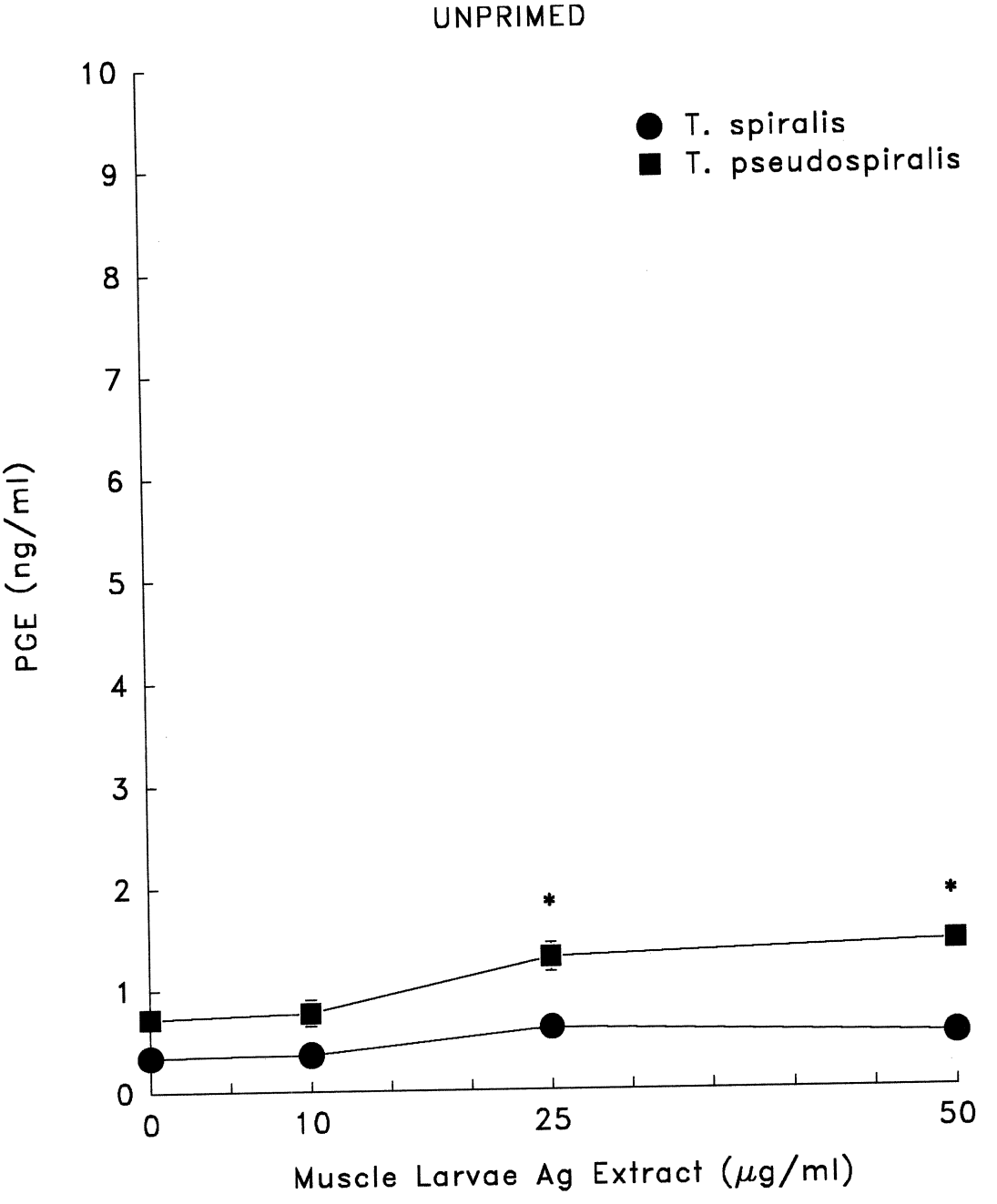

UNPRIMED
T. spiralis
T. pseudospiralis
PGE (ng/ml)
10
9
8
7
6
5
4
3
2
1
0
*
*
0
10
25
50
Muscle Larvae Ag Extract (μg/ml)

Figure 23

Peritoneal cells collected from BCG cell wall-primed mice were cultured with 0, 10, 25, and 50 μg/ml concentrations of *T. spiralis* or *T. pseudospiralis* adult antigens. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE of at least two experiments. A * above the symbol indicates a significant difference between *T. spiralis* and *T. pseudospiralis* values ($P<0.05$).

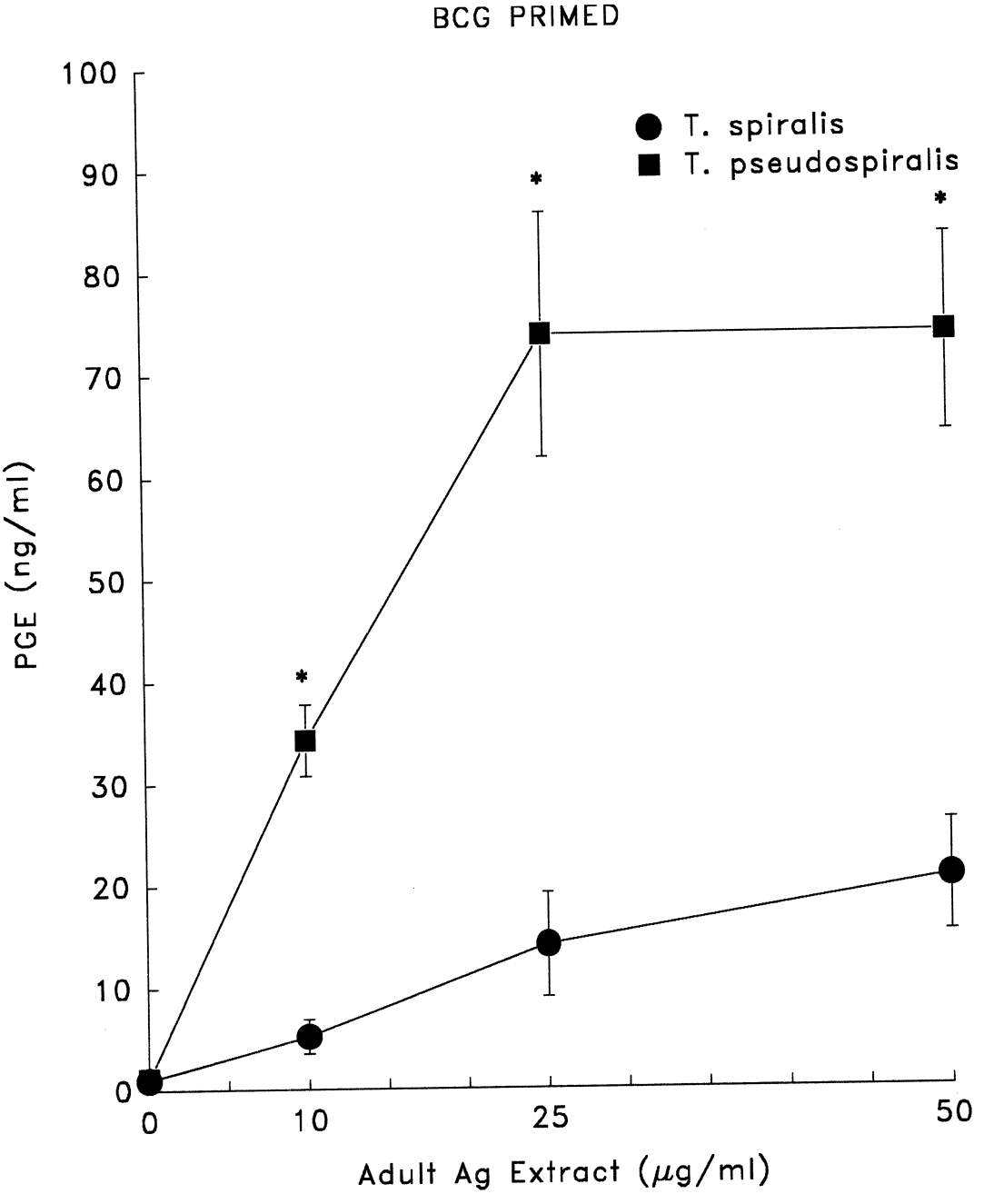

BCG PRIMED
T. spiralis
T. pseudospiralis
PGE (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
0
10
25
50
Adult Ag Extract (μg/ml)
*
*
*

Figure 24

Peritoneal cells collected from BCG cell wall-primed mice were cultured with 0, 1, 5, and 10 μg/ml concentrations of *T. spiralis* or *T. pseudospiralis* newborn larvae antigens. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE of at least two experiments.

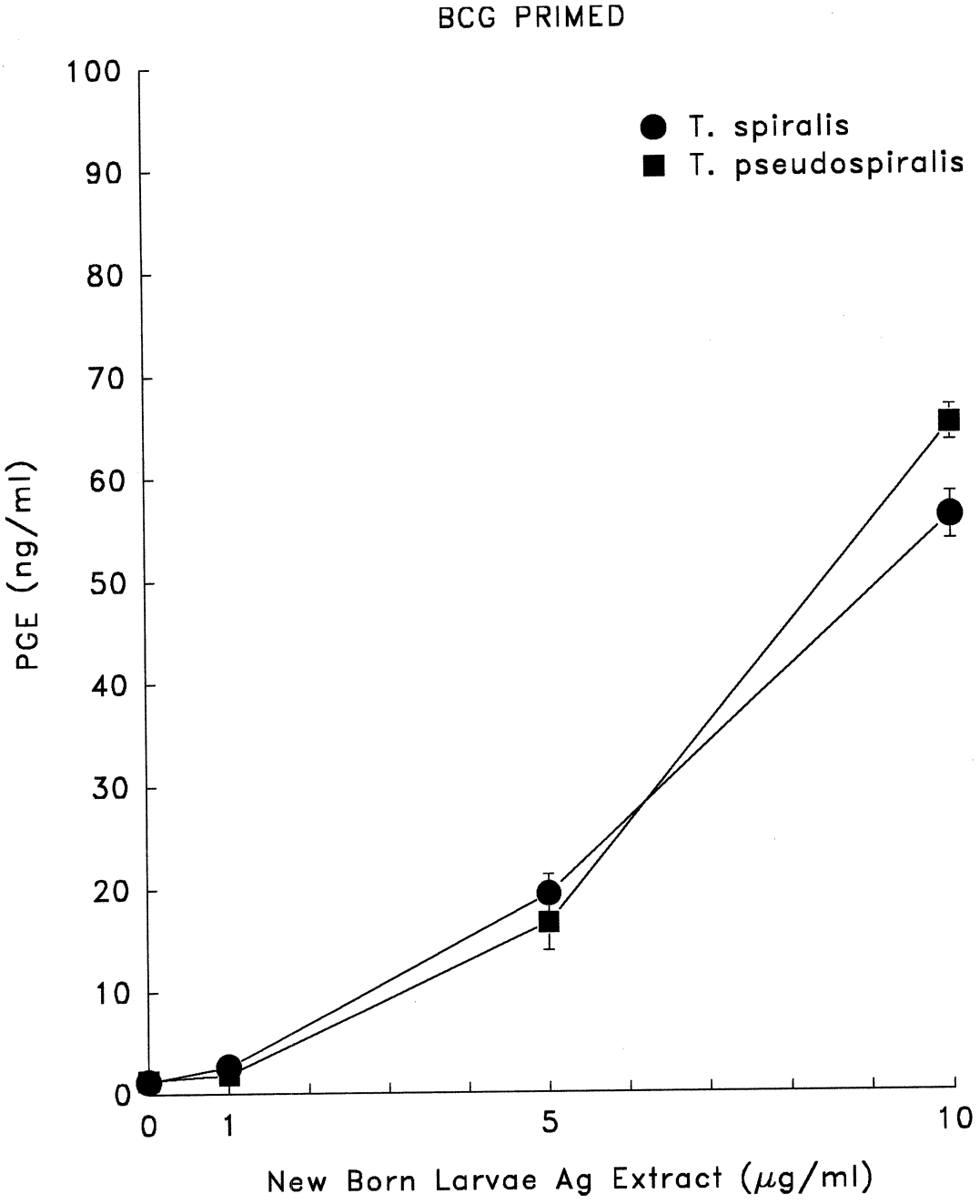
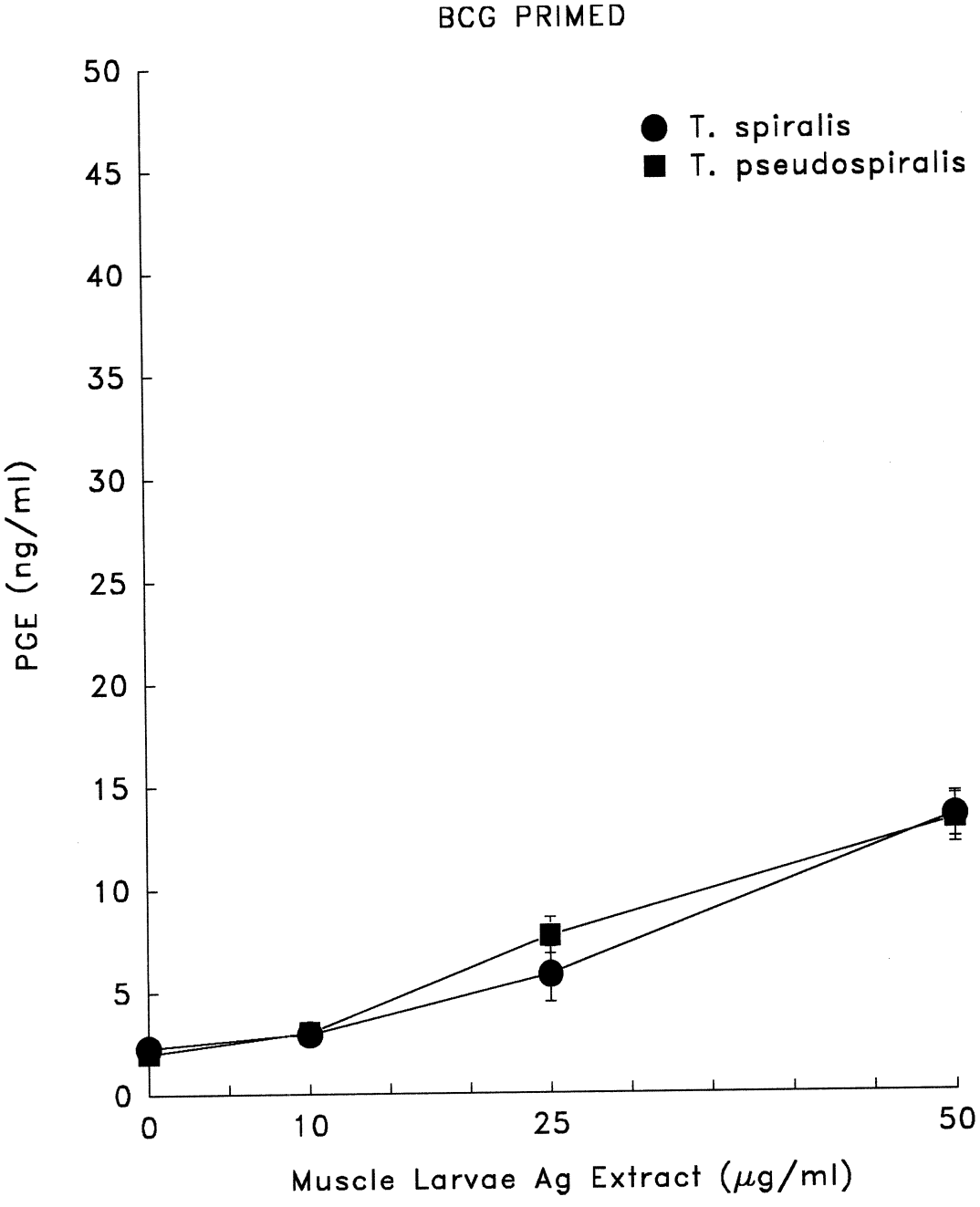

BCG PRIMED
T. spiralis
T. pseudospiralis
PGE (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
0
1
5
10
New Born Larvae Ag Extract (μg/ml)

Figure 25

Peritoneal cells collected from BCG cell wall-primed mice were cultured with 0, 10, 25, and 50 μg/ml concentrations of *T. spiralis* or *T. pseudospiralis* muscle larvae antigens. After 24 hours the culture supernatants were harvested and the PGE extracted. The quantity of PGE was determined by radioimmunoassay. The symbols represent the mean ± SE.


BCG PRIMED
T. spiralis
T. pseudospiralis
PGE (ng/ml)
50
45
40
35
30
25
20
15
10
5
0
0
10
25
50
Muscle Larvae Ag Extract (μg/ml)

CHAPTER IV

DISCUSSION

Substantial differences exist between immune responses elicited in mice infected with *T. pseudospiralis* when compared with those occuring in animals infected with *T. spiralis*. The aggregate knowledge available on the nature of immune responses in *T. spiralis* infections is extensive. However, the nature of immune responses in *T. pseudospiralis* infections are undefined. Furthermore, the role of the macrophage in immune responses to either parasite is unclear. In the present study, cell counts, differential counts, and monoclonal antibodies were used to elucidate the role of macrophages during these *Trichinella* infections.

The cellular dynamics of peritoneal cells during *Trichinella* infections are not well documented. Thus, the magnitude and pattern of cellular response in the peritoneal compartment was characterized. In this study peritoneal cell numbers in mice infected with *T. spiralis* or *T. pseudospiralis* reach a maximum around the time of worm expulsion from the gut, which is similar to results reported in an earlier study (Dixon, 1987). These data correlate with the occurence of peak inflammation in the gut tissue surrounding the adult worms (Larsh and Race, 1975). The intensity of this inflammatory process causes the distribution of cell types to change during the infection sequence. It is noteworthy, that the proportion of peritoneal macrophages increases during the intestinal phase of these infections, again correlating with peak inflammation in the gut.

An explaination for the increase in peritoneal macrophages during the early intestinal phase is that parasite antigens responsible for recruiting immune cells to the site of infection also reach the peritoneal space. This is supported by the prescence of newborn larvae in the peritoneum of infected mice (G. Dixon, unpublished data). Furthermore, significantly greater numbers of peritoneal macrophages were recovered from *T. pseudospiralis*-infected animals, than were recovered from *T. spiralis*-infected animals late in the intestinal phase of these infections. This finding indicates that the host inflammatory response is different during *T. pseudospiralis* infections, and also suggests that macrophages may be specifically modulated by this parasite.

No other remarkable trends in cell distribution were noted, except for the characteristic increase in eosinophils at the end of the intestinal phase. There were no significant differences between the eosinophil numbers from *T. spiralis*-infected mice and *T. pseudospiralis*-infected mice. This is unlike the results reported in an earlier study (Dixon, 1987), where the numbers of peritoneal eosinophils recovered on day 12 PI from *T. spiralis*-infected mice was substantially higher than those recovered from *T. pseudospiralis*-infected mice. The earlier study used higher infective doses of 1000 *T. spiralis* larvae or 2000 *T. pseudospiralis*-infective larvae, versus the lower doses of 500 *T. spiralis* or 500 *T. pseudospiralis* used in this study. This indicates that higher doses of *T. pseudospiralis* are more effective in reducing eosinophil numbers, and in contrast, higher *T. spiralis* doses enhance eosinophil numbers. This *T. spiralis* infective dose-effect on eosinophil numbers was documented by Ismail and Tanner (1972).

Monoclonal antibodies against macrophage surface markers were used to delineate the subpopulation of macrophages present in the peritoneum of control, BCG cell wall primed, *T. spiralis*-infected, and *T. pseudospiralis*-infected mice. The integrity of the monoclonal antibody reagents used were validated by using a FALS-IGFL dual parameter histogram. M1/70 and B21-2 labelled resident peritoneal macrophages (Springer et al., 1979; Steinman et al., 1980), whereas M3/38 failed to label resident peritoneal macrophages which agrees with the findings of Ho and Springer(1982). On the other hand, thioglycollate-elicited macrophages were labelled by M3/38, and this is supported by the findings of Ho and Springer, 1982. These surface markers were also tested with BCG cell wall-elicited macrophages. This subsequently revealed that resident peritoneal macrophages express a similar pattern of fluorescence when compared to BCG cell wall-elicited macrophages. The specific induction of Mac-2 expression on thioglycollate-elicited macrophages and a lack of Mac-2 expression in macrophage populations induced by BCG cell walls is supported by the observations of Ho and Springer (1982).

Peritoneal macrophage subpopulations from *T. spiralis* or *T. pseudospiralis*-infected mice followed the same general trend. The absolute number of cells expressing Mac-1, Mac-2, and Ia antigens increased through day 12 PI. This results from the increasing proportion of macrophages with concomitant increases in the total peritoneal cell number. Interestingly, absolute Mac-1 expression is consistently lower than the absolute macrophage count. Since the Mac-1 and Ia values correlate, these estimates of the macrophage numbers are more accurate than the differential count. Although the Mac-2 expression increased during the intestinal phase of these infections, the proportion of

macrophages expressing Mac-2 remained low. This indicates that the most intense interaction between the parasite and host macrophages is occuring with a Mac-1$^+$, Mac-2$^-$, Ia$^+$ subpopulation. However, it remains unclear if relatively small numbers of Mac-2 $^+$ macrophages can produce sufficient quantities of immunosuppressive factors to have physiological consequences. Further study is required to determine the effects of low numbers of Mac-2$^+$ macrophages on the immune response. Furthermore, the immune suppression exhibited during *T. pseudospiralis* infections does not appear to be related to a lack of Ia expression by macrophages, although these data do not preclude the possibility that there is a functional deficit associated with Ia actions.

The immunological consequences of elevated levels of PGE are substantial. Every branch of the cell-mediated immune response is suppressed by E series prostaglandins. In the present study the dose effect of LPS on control and BCG cell wall-primed macrophages was investigated with respect to macrophage ability to produce PGE. The data collected reveal that macrophages primed with BCG cell walls in vivo produce 2-fold greater levels of PGE at all doses of LPS. This indicates a synergistic relationship between BCG priming and LPS exposure. BCG antigens are well known for their ability to potentiate immune responses (Molinari et al., 1975). In this case exposure to BCG cell walls upregulate the pathways for PGE production. Similarly, *Trichinella* antigens may potentiate immune responses, thus enhancing PGE production above the levels seen with naive macrophages.

Peirtoneal macrophages recovered from *T. spiralis* or *T. pseudospiralis*-infected mice failed to produce significantly greater levels of PGE than control mice after LPS stimula-

tion. Interestingly, an earlier study (Dixon, 1987) demonstrated that remarkably higher levels of PGE were produced by macrophages recovered from both parasite infections than from control macrophages. The current data reflect findings similar to those previously discussed with regard to eosinophilia. The lower dose of worms used in the present study may not provide adequate exposure of peritoneal cells to parasite antigens. Mouse peritoneal lavage fluid recovered from mice also failed to show significantly increased levels of PGE; however, there were some noteworthy differences. For example, the computed significance level for the comparison of *T. spiralis* day 4 PI PGE production with *T. pseudospiralis* day 4 PI PGE was 0.10. Likewise, the significance level was 0.08 when the control PGE value was compared to the *T. pseudospiralis* day 4 PI PGE value. This indicates that some interesting differences exist in the *in situ* production of PGE early during the intestinal phase of *T. pseudospiralis* infections. These data correlate well with the pattern of PGE production by peritoneal macrophages recovered from these *Trichinella* infections and stimulated with LPS on day 4 PI. Moreover, these data agree with the previous observations that *T. pseudospiralis* PGE levels are elevated early in the intestinal phase of infection (Dixon, 1987).

Soluble antigen extracts of *T. spiralis* and *T. pseudospiralis* adults, newborn larvae, and muscle larvae were incubated with peritoneal macrophages recovered from uninfected mice in order to determine the immunomodulatory potential of these antigens. The present study reveals that *Trichinella* antigens possess direct macrophage stimulatory activity as indicated by PGE production. Interestingly, *Trichinella* newborn larvae extracts possessed the greatest macrophage stimulatory activity, which was closely followed by

adult extracts, and the lowest activity was induced by the muscle larvae extracts. These observations correlate with the pattern of chemotactic response of neutrophils to *Trichinella* antigens (Shupe and Stewart, 1991). Furthermore, peritoneal macrophages recovered form BCG cell wall-primed mice produced 2-3 fold more PGE when incubated with *Trichinella* adult and muscle larvae antigen extracts. Thus BCG cell wall priming and *Trichinella* antigen extract exhibit synergism in their action. In contrast, PGE production induced by BCG cell wall-primed macrophages and newborn larvae antigens failed to show synergism. One possible explaination is that the newborn larvae extracts, which are highly potent in driving macrophage PGE production, may be capable of causing macrophages to synthesize PGE at the maximum rate.

Some remarkable differences were observed between macrophage PGE response to *T. spiralis* and *T. pseudospiralis* antigen extracts. *T. pseudospiralis* adult antigen extracts induced at least four fold greater PGE production by peritoneal macrophages in both untreated and BCG cell wall treated groups, than did adult antigen extracts of *T. spiralis*. This enhancement of macrophage PGE production by *T. pseudospiralis* adult antigens probably contributes to the immune suppression seen during the intestinal phase of these infections. Among the parasite extracts examined, the adult and newborn larvae extracts of *T. pseudospiralis*, and the newborn larvae extracts from *T. spiralis* demonstrate the best potential for parasite factors that alter host immunity. Although *T. pseudospiralis* muscle larvae extracts induce more PGE production than *T. spiralis* muscle larvae extracts, their highest activity was always lower than the activity of the adult and newborn larvae

from either parasite. Consequently, the muscle larvae do not contribute macrophage-mediated suppression seen early in *T. pseudospiralis* infections.

In summary, the results of this study clearly establish that *Trichinella* antigens directly influence macrophages to produce immunosuppressive agents such as PGE. The soluble products released by *Trichinella* adults are capable of modulating macrophage production of PGE, and consequently may alter the host immune response during the intestinal phase of these infections. Furthermore, the soluble extracts of adult *T. pseudospiralis* induce more macrophage PGE production than *T. spiralis* adult extracts, thus providing evidence supporting the conclusion that *T. pseudospiralis* adults mediate the immune suppression seen during the intestinal phase of *T. pseudospiralis* infections. Likewise, the high levels of macrophage PGE induced by *Trichinella* newborn larvae probably assists these migratory larvae in evading the host immune responses which may damage or destroy the newborn larvae before they establish the muscle phase of infection. The muscle lavae extracts of *T. pseudospiralis* induce more PGE production by macrophages than *T. spiralis* muscle larvae extracts, and thus may contribute to the mechanisms of immune evasion and immune suppression employed by this parasite during the muscle phase of infection. Infection with *T. pseudospiralis* also enhances macrophage numbers above the levels typically seen in *T. spiralis* infections. The combination of enhanced macrophage numbers and the sensitivity of these macrophages to *T. pseudospiralis* extracts indicates that *T. pseudospiralis* employs a novel mechanism of mediating immunosuppression. These data contribute additional insights into the complex nature of the *T. pseudospiralis* host-parasite relationship.

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