The helminth parasite, *Trichinella pseudospiralis*, elaborates two T cell immunomodulatory substances. The intestinal stage of the parasite releases an interleukin 10-like cytokine substance, TP10, that directly suppresses Th1 responses and dampens inflammation at the site of infection in the small intestine. The tissue invasive newborn larvae elaborate a substance, TP1, that induces lymphoid and non-lymphoid cells to secrete enormous quantities of interleukin 2-like activity. TP1-induced IL-2-like activity causes dramatic proliferation of an IL-2 dependent cell-line (HT2 cells) and an increase in release of cytokines by cells of the Th1 subset (IL-2, interferon gamma and IL-3). During the normal course of infection with *T. pseudospiralis*, TP1 is initially released locally in the small bowel of the host and causes a dramatic activation of Th1 cells in the mesenteric lymph nodes with generation of a severe enteritis that expels the adult worms. Systemic distribution of the newborn larvae (the source of TP1), is accompanied by a dramatic increase in plasma levels of Th1 cytokines (predominantly IL-2), which feedback on the hypothalamic-pituitary-adrenal axis causing a profound increase in plasma corticosterone and a systemic suppression of cellular immunity.
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DESCRIPTION

IMMUNOMODULATORY TRICHINELLA SUBSTANCES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the fields of immunotherapy and immunosuppression. The invention is directed to the identification of novel substances with profound immuno-modulatory effects. More particularly, it concerns the identification of novel factors obtainable from the Trichinella sp. during mutually exclusive stages of its life cycle that directly exert an effect on T cells without countervailing side effects. The invention further concerns the use of these novel substances and their analogues for immunotherapy and methods for identifying and purifying said components.

2. Description of the Related Art

The phenotypically similar group of helminth parasites collectively known as the Trichinella sp. are responsible for widespread morbidity and mortality. Helminth parasites display a remarkable capacity to escape immunological destruction and may reside in their vertebrate hosts with relative impunity, sometimes for the life of the host. Trichinella pseudospiralis is an especially interesting example of a metazoan parasite that employs several strategies for avoiding immune rejection. Unlike muscle larvae of a closely related species, Trichinella spiralis, muscle larvae of T. pseudospiralis are not enclosed by a collagenous capsule and, are exposed to host immunological effector elements.
The life cycle of *T. pseudospiralis* begins with the consumption of vertebrate muscle containing infective larvae. The intestinal phase of infection is initiated when larvae liberated from host muscle in the stomach enter the small intestine as preadults which mature to the adult stage within 24-48 hrs. The parenteral phase of infection begins when adult female worms release newborn larvae which enter the lymphatics and blood vessels and are distributed throughout the body of the host. The newborn larvae enter striated muscle fibers and reside as intracellular muscle larvae. Unlike the muscle larvae of *T. spiralis*, those of *T. pseudospiralis* are not enclosed by a protective collagenous capsule in host muscle. Thus the life history of *T. pseudospiralis* has three distinct stages: 1) preadult/adult worms, 2) newborn larvae, and 3) muscle larvae.

The ability of *T. pseudospiralis* to escape immunological attack is associated with a profound suppression of antigen-specific and nonspecific inflammatory responses. Mice infected with *T. pseudospiralis* develop significantly elevated levels of plasma corticosterone that coincide with severe inhibition of delayed-type hypersensitivity (DTH), contact hypersensitivity, and granuloma responses (Stewart, et al., (1985, 1988, 1989, 1991)). The remarkable downregulation of host inflammatory responses in infected mice, in light of elevated serum IgE antibodies (Stewart, et al., (1989)), suggests that *T. pseudospiralis* infection selectively regulates T cell-dependent immune responses.

In addition to systemic suppression of inflammatory responses, *T. pseudospiralis* utilizes antigenic mimicry to avoid immunological recognition. The muscle larvae of *T. pseudospiralis* express host-like asialo ganglio-N-tetraosylceramide (asialo GM1) antigen, which is believed
to provide antigenic camouflage for the nonencapsulated larvae (Niederkorn, et al., (1988), and Stewart et al., (1989)). However, limited studies on this particular organism have been conducted, given the preeminence of the closely related causative agent of trichinosis, *Trichinella spiralis*.

It is generally accepted that all parasites must modulate host immunity in order to survive in an immunocompetent host. The immunoevasive strategies employed by parasites vary greatly and are a very popular subject for investigation. They provide information about the survival methods of the parasite and how the immunological balance may be tipped in favor of, or against, the host. However, major questions remain regarding the vertebrate immune response to helminth parasites, such as how it is controlled and what are the limitations that allow the parasite to successfully infect and propagate within the host.

*Trichinella pseudospiralis* exists in three different stages during its stay in a vertebrate host. The host response against this parasite would be expected to be very severe. However, this conclusion is drawn from studies on other species of the genus which have identical life cycles and infect similar hosts. Although this parasite appears vulnerable to immunological destruction, it survives because the host does not respond in the expected fashion. The present inventor conducted extensive studies of this organism to determine how it evades destruction by host immune response. The parasite suppresses the inflammatory response against itself and against any non-self organism or material present in the host during infection with *T. pseudospiralis*. 
In this host-parasite system, the newborn larval stage (which migrates systemically from the intestine to the muscle), and the muscle-stage parasite (which must exist for long periods of time in host muscle, in some cases for the life of the host) must both avoid non-specific inflammatory responses by the host. During its stay in host muscle the parasite continuously migrates within and between muscle fibers, causing low level damage to host tissues. Such activity would normally attract enormous numbers of host inflammatory cells. By contrast, in infections involving other members of this genus the muscle larvae do not migrate and are enclosed within a capsule which affords them protection from immunological attack by the host. For *T. spiralis* an intense influx of inflammatory cells into infected host muscle occurs in response to the mechanical and chemical insults wrought by the encapsulated muscle larvae.

The present inventor has shown that infection with *Trichinella pseudospiralis* is accompanied by high levels of plasma corticosterone (Stewart, *et al.*, 1988). It is well documented that several key cytokines released by macrophages and T cells have the potential for modulating immune response through their secondary role as chemical communicators with the hypothalamic-pituitary-adrenal (HPA) axis. That is, IL-1, IL-2 and other cytokines have been shown to influence pituitary or adrenal function in a way that leads to an increase in corticosteroid release.

The immunosuppressive properties of corticosteroids are well documented. Thus, while cytokines operate to regulate host immune responses by their direct influence over the functions of immunological cells, they may also bring about a down-regulation of immune response indirectly through their influence over the HPA axis. Currently, the mechanisms responsible for this effect are
unknown. One of the possible explanations for the elevation of plasma corticosterone in animals infected with Trichinella pseudospiralis has been offered by Stewart et al. (1988). It was proposed that the parasite may be inducing the release of at least one cytokine which feeds back on the HPA axis, bringing about an elevation in plasma corticosterone and a suppression of the non-specific inflammatory response.

Studies of the closely related T. spiralis, have extensively documented the cellular immune response to Trichinella infection. Susceptibility of inbred hosts correlates with T helper-2 (Th2) driven immune responses, as characterized by the secretion of interleukins (IL) - 3, -4, -5, -9, and -10. The primary functions of IL-10 are to antagonize the effects of T helper-1 (Th1) cells, and promote Th2 driven B cell help. T. spiralis is a strong inducer of the Th2 phenotype by, for example, inducing the secretion of IL-10 by T cells, thereby skewing the immune response toward the susceptible phenotype.

A great amount of attention is currently focused on using cytokines to treat a wide variety of human diseases, including cancers, AIDS, infections with cytomegalovirus, herpes and a long list of viral and parasitic disease agents. Likewise, cytokines that ameliorate the effects of autoimmune diseases, such as rheumatoid arthritis, pemphigus, systemic lupus erythematosus, and others are highly coveted. Unfortunately even highly purified recombinant human cytokines may be highly toxic and with a wide variety of detrimental side-effects. A need remains for agents useful in immune intervention that are not harmful to the host.
One of the problems with this therapeutic approach is that most of the immune-related cytokines, including Lymphotoxin, Interferon-γ, Tumor Necrosis Factor, IL-1, IL-2, and IL-10, have many adverse side-effects in animals and humans. These side effects have precluded their widespread use as therapeutic agents. The generation of a cytokine or cytokine-like substance which has reduced toxicity towards human cells is necessary for the development of greatly improved methods for clinical immunotherapy. In particular, the identification of an agent, such as that of the present invention, that directly exerts its action on T cells without the toxic side-effects generally associated with the systemic use is important in the development of new therapeutic strategies with wide ranging applications.

**SUMMARY OF THE INVENTION**

The present invention, in a general and overall sense, concerns novel *T. pseudospiralis* components applied to clinical and experimental immunology. It also seeks to overcome one or more of the drawbacks inherent in the prior art by providing methods for the purification of novel substances having cytokine activity and compositions for use in human immunotherapy with reduced side effects. The present invention relates to the unexpected discovery of potent and selective immunomodulatory substances produced during infection with the parasite, *T. pseudospiralis*.

Infection with this parasite and administration of certain extracts and excretory/secretory products generally suppress vertebrate inflammatory response to self and nonself antigens. As used herein the term, "exudate" will be used interchangeably with the terms "secretion/excretion" to describe the release, natural or artificial of the present invention. This is
demonstrated using a number of different in vivo and in vitro model systems for inflammation. Vertebrate immune responses are orchestrated by macromolecular messengers elaborated by immune cells. These macromolecules, the cytokines, comprise a system of molecular communications between cells which have specific effects in directing cellular and humoral responses, for example, against non-self organisms and material. Interleukins have far-reaching effects on host cellular and humoral immunity and their regulatory networks. These pluripotent effects are well known to those versed in the art of immunology.

The present invention concerns two substances in the exudates of two different stages in the parasite's life cycle that modulate immunity. As used herein, the term "excretory/secretory", ("excretory" or "secretory"), or "exudate", are used to define nematode parasite substances that are found in the extracts or supernatants derived from the helminth parasite Trichinella pseudospiralis. These exuded products or substances may be derived from, for example, extracts of nematode infected tissue, the supernatants of nematode-containing media or humors, or by other methods commonly known to those of skill in the art, in light of the present disclosure. Those of skill in the art can use the methods and examples described herein to identify and purify substances with activities similar to those described in the present invention from T. pseudospiralis or other members of the Trichinella sp..

The present inventor has shown that T. pseudospiralis exudes a substance that has interleukin-10-like activity. The IL-10-like substance isolatable and purifyable from exuded products of the Trichinella sp., is hereinafter referred to as TP10. TP10 has an approximate molecular weight of between 100 and 300kD in one form, and is highly specific in its
effects. However, it is, of course, generally understood by those of skill in the art that the migration of a polypeptide can vary with different conditions of SDS/PAGE (Capaldi et al., 1977). It will therefore be appreciated that under differing electrophoretic conditions, the molecular weight assignments quoted above may vary, for example molecular weight may vary due to the formation of multimeric complexes that have similar activity to the monomeric form.

TP10 is generally obtained from the preadult stage of T. pseudospiralis and directs the activation or "skewing" of T helper cells toward a differential ratio of T cell subsets called Th2 cells and away from the Th1 phenotype. The terms, Th1 and Th2, as used herein, describe distinct T cell phenotypes generally associated with inflammatory and B cell helper phenotypes, respectively, and are well known to those skilled and versed in the art. Th1 cells promote inflammatory response and the bias induced by the parasite exudate against this subset is likely to cause the remarkable suppression of inflammation during the intestinal phase of infection, as disclosed in the present invention.

A preferred embodiment of the invention is a purified Trichinella-derived substance (TP10) having certain immunomodulatory characteristics of IL-10. These characteristics include a Th1 immunosuppressive effect. As used herein, "Th1 immunosuppression" is defined as the inhibition or termination of an inflammatory immune response. In a preferred embodiment this substance is defined further as derived from Trichinella pseudospiralis, with a substance derived from a pre-adult larvae of Trichinella pseudospiralis being more preferred.
A related embodiment is a purified IL-10-like substance having the following characteristics; isolatable from pre-adult Trichinella exudates in a conditioned media; having a molecular weight of between 100-300 kD; and being immunosuppressive to T cells of the Th1 phenotype. As used herein the term, "conditioned media" is used to describe a medium, extract, or humor, wherein the nematodes have been allowed to incubate and have exuded substances in accordance with the present invention. The term "purified" as used herein, is intended to refer to a Trichinella-derived cytokine-like or cytokine inducing substance that is obtainable from Trichinella sp. organisms, wherein the composition comprises a substance that is purified to any degree relative to its naturally-obtainable state, i.e., in this case, relative to its purity within a member of the Trichinella sp.. In a preferred embodiment TP10 is derived or purifiable from the preadult stage of members of the Trichinella sp.. Also in a preferred embodiment (described below) is TPi, which is derived or purifiable from the newborn larvae of members of the Trichinella sp.. In most preferred embodiments TP10 and TPi are derived or purifiable from T. pseudospiralis. A purified "TP10" or "TPi", therefore, refers to a substance free from the environment in which it may naturally occur in intact cells, e.g., free of infective Trichinella sp. organisms.

Another embodiment of the invention is a method of obtaining a purified IL-10-like substance (TP10) from Trichinella pseudospiralis comprising the steps of; obtaining Trichinella pseudospiralis organisms; incubating said organisms under conditions facilitating the exudation of a IL-10-like substance (TP10), and purifying a Trichinella IL-10-like substance (TP10) from said exudate containing incubate by separating intact organisms or fragments thereof. In a preferred embodiment
the purifying step is accomplished by separating intact organisms or fragments thereof through a 0.2μm filter. In a more preferred embodiment the purification step is accomplished by ultrafiltration with a molecular weight cut-off of between about 10 kDa to 100 kDa. In an even more preferred embodiment purification is accomplished by ultrafiltration with a molecular weight cut-off of about 10 kDa.

The present invention also comprises an antibody having specific binding affinity for an IL-10-like factor produced by a member of the Trichinella sp.. A related embodiment of the invention is a method of purifying an IL-10-like substance (TP10) having the ability to stimulate T cells toward a Th2 phenotype and away from a Th1 phenotype comprising; obtaining an exudate from freshly isolated or cultured nematodes, and retrieving an IL-10-like substance (TP10) from said exudate with an antibody having a specific binding affinity for the IL-10-like substance in accordance with the present invention.

Newborn Trichinella pseudospiralis larvae exude a separate substance that stimulates host cells, e.g., fibroblasts and T cells, to secrete large amounts of a molecule with potent cytokine-like activity. The molecule released by fibroblasts and T cells stimulates proliferation of HT2 cells (IL-2 dependent cell line) in vitro. Surprisingly, the released molecule does not cross-react in an ELISA for IL-2. However, the release of both gamma interferon and IL-2 are dramatically increased in cultures of mouse spleen cells and mesenteric lymph node cells exposed to the parasite molecule in vitro. Thus, the induced molecule acts as a potent Th1 mitogen. Moreover, injection of the parasite molecule into animals is accompanied by Trichinella spiralis-induced enteritis, and interstitial cells in
muscle infected with *T. pseudospiralis* react strongly
with a probe for IL-2 mRNA. The active cytokine-inducing
substance, hereinafter referred to as TPi, can be
isolated and purified from the parasite’s exuded
products. TPi is characterized as having an approximate
molecular weight of between 30 and 50 kD and which
induces cells, such as fibroblasts and T cells, to
release a cytokine-like molecule. The release of
elevated levels of this molecule induced by the products
of the *Trichinella* sp. are likely to cause the adrenals
to release increased amounts of corticosterone, raising
host plasma levels of this steroid thereby inducing the
systemic suppression of inflammatory responses to both
self and non-self antigens, including the parasite.

Studies on animals infected with *T. pseudospiralis*
clearly demonstrate that this parasite induces very low
levels of pathology in the host, and an absence of the
changes seen in the infected host mimics the side-effects
normally associated with cytokine therapy. *In vivo* and
*in vitro* studies using products from this parasite show
that none of the side-effects normally seen in cytokine
immunotherapy. Furthermore, the inventor routinely uses
TP10 and TPi as substitutes for their respective
cytokines for use in *in vitro* assays, as described
herein. The function of the IL-10-like substance (TP10)
and of the cytokine-inducing substance (TPi) of parasite
origin are also shown to be very specific and limited in
their function.

These two substances are of great value for
immunological manipulation, and also represent a
revolutionary approach to immunotherapy in treating the
enormous array of pathologies where inflammation
participates in pathogenesis. This includes a wide
variety of autoimmune and inflammatory diseases or
tissue-graft rejection. TP10 may also be useful in the
treatment of toxemias induced by bacterial infections in which tumor necrosis factor and/or IL-1, are generally elevated under the influence of Th1 subset of lymphocytes. TP10 may also be used to skew the immune response away from a Th1 phenotype to a Th2 phenotype, thereby enhancing Th2 functions such as B cell help. TPi, depending on the amount administered to animals, has different effects on the immune status of treated animals.

For example, during infection with *T. pseudospiralis*, when newborn larvae are deposited by adult female worms in the intestinal wall, release by the larvae of TPi has a local effect on immunological events in the intestine. This consists of an activation of T cells of the Th1 subset in the mesenteric lymph nodes, which is accompanied by the development of expulsion of adult worms, an event necessary to protect the host from superinfection. Later, as newborn larvae are distributed systemically, an activation of T cells of the Th1 subset is apparent in the spleens of infected animals, followed by a rise of Th1 cell cytokines in host plasma and peritoneal washings. These events are accompanied by a rise in plasma corticosterone levels and a systemic, non-specific suppression of inflammatory response. After this point during infection, spleen cells recovered from *T. pseudospiralis*-infected mice and stimulated in vitro with Con A, release significantly lower levels of cytokines than cells from uninfected animals. Thus, TPi can, at low levels, induce activation of Th1 cells (injection of TPi into Chinese hamsters early during the intestinal phase of infection with *T. spiralis* causes an increase in enteritis with rapid expulsion of the adult parasite) or at high and continuous doses induce a suppression of systemic inflammation via cytokine feedback on the hypothalamic-pituitary-adrenal axis such as that seen during the normal course of infection with
this parasite. Through their cytokines, Th1 cells orchestrate a wide variety of essential immunological events. In an immunosuppressed host, TPI may be used to stimulate release by non-lymphoid cells or undamaged T cells of the potent Th1 cell mitogen, dramatically boosting Th1 cell activation and proliferation and reversing the immunosuppression.

The term "Trichinella derived substances" as used herein, refers to a composition of the present invention (TP10 or TPI) that has been subjected to fractionation to remove various non-active components, and which composition substantially retains its immunomodulatory activity. Where the term "purified" is used, this will refer to a composition in which TP10 or TPI cytokine-like activity is obtained by purifying the active fractions of exuded products away from non-active components, such as whole organisms.

A preferred embodiment of the invention is a purified Trichinella-derived substance eliciting release of an IL-2-like substance by eukaryotic cells. The substance is defined further as being derived from Trichinella pseudospiralis, with purification from the newborn stage of Trichinella pseudospiralis being most preferred. The IL-2-inducing substance may be further defined as: being isolatable from exudates of newborn Trichinella conditioned media, having a molecular weight of 30-50 kD, and being capable of inducing secretion of IL-2 by eukaryotic cells.

A related embodiment is a method of obtaining a purified IL-2-inducing substance comprising the steps of: obtaining Trichinella pseudospiralis-organisms; incubating said organisms under conditions facilitating the exudation of an IL-2-inducing substance, and purifying a Trichinella derived IL-2-inducing substance.
from said incubate by separating intact organisms or fragments thereof. In a preferred embodiment the purifying step is accomplished by separating intact organisms through a 0.2μm filter. In a more preferred embodiment the purifying step is accomplished by ultrafiltration with a molecular weight cut-off of between about 10 kDa to 30 kDa. In another preferred embodiment the purifying step is accomplished by ultrafiltration with a molecular weight cut-off of about 10 kDa.

Another embodiment of the present invention is an antibody having specific binding affinity for a IL-2-inducing activity substance produced by a member of the Trichinella sp.. A related embodiment is a method of purifying an IL-2-inducing substance able to stimulate eukaryotic cells to secrete an IL-2-like activity, comprising; obtaining an exudate from freshly isolated or cultured Trichinella and retrieving an IL-2-inducing substance from said exudate with an antibody having specific binding affinity for said IL-2-like inducing substance.

Various methods for quantifying the degree of purification of the cytokine-like substances of the present invention are known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the number of polypeptides within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a T. pseudospiralis cytokine-like fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial nematode extract or supernatant, and to thus calculate the degree of purity.
The actual units used to represent the inhibitory activity of TP10 and IL-2-inducing activity of TPi will, of course, be dependent upon the particular assay technique chosen to follow the purification. As discussed above, the present inventor prefers to use an assay based upon the inhibition or immunosuppression of immune cell responses both in vitro and in vivo. Activity in the assay as used herein is defined as the immunosuppressive activity of TP10 required to cause a reduction in cytokine release by Th1 cells. However, using other assays, the definition of a unit of activity would naturally vary. It may be assessed as, for example, the activity to other Th1-like cells or stimulation of Th2 cells.

For assessing the purification and purity of TPi, the present inventor prefers to use an assay based upon: 1) secretion of a Th1-stimulating molecule by fibroblasts; 2) secretion of T cells in vitro; or 3) stimulation of natural killer cells in vivo or in vitro. Activity in the assay, as used herein, is defined as the extent of stimulation required for cytokine release by Th1 cells or release of Th1-stimulating molecule by NIH 3T3 cells in vitro. However, using other assays, the definition of a unit of activity would naturally vary. It may be assessed as, for example, the activity to stimulate other cells capable of producing cytokines or by the stimulation of Th1 cells.

In one embodiment, to prepare purified TP10 in accordance with the present invention one obtains muscle tissue from mice convalescing with the muscle stage of Trichinella pseudospiralis. The muscle tissue is homogenized, e.g., in 1% pepsin/HCl for isolation of larvae, and the resulting homogenate is incubated at 37°C for 1 hr. Incubates are allowed to sediment and the larvae are washed twice, sedimented, placed in glass
incubation chambers in medium as described herein and incubated for 48-72 hr at 37°C. Larvae are removed by centrifugation at 1000 rpm, and the supernatant is passed through a 0.22 μm filter, and concentrated to 5X using a 10kD membrane in an ultrafiltration apparatus.

To prepare a purified TPi cytokine-inducing substance in accordance with one embodiment of the present invention one isolates adult worms from the intestines of rats infected with *T. pseudospiralis* isolated from mouse muscle by conventional methods (e.g., Pepsin-HCl). Briefly, the entire small intestine of each infected animal is removed, washed, and adult worms allowed to migrate out of the intestinal wall into sterile saline. The preferred animals for raising nematodes are rats, mice, and rabbits. Intestines and debris are removed by passing the worm suspension through a series of sieves and worms are washed three times by centrifugation. The isolated worms are placed in medium and incubated at 37°C for 24 hr in a water bath, allowing adult worms to release newborn larvae. Following incubation, adult worms are removed by passing the worm suspension through a series of sieves. Recovered newborn larvae are washed 5 times in fresh medium and incubated in fresh medium for 48 hr at 37°C. Following incubation, newborn larvae are removed by centrifugation, and excretion/secretion (ES) products are obtained and purified as described for TP10. The methods described are conventional and well known to those of skill in the art.

Generally, purified *Trichinella pseudospiralis* derived compositions have been subjected to fractionation to remove various non-active substances such as other cell components. Various techniques suitable for use in further purification are well known to those of skill in the art. These include, for example, precipitation with
ammonium sulphate, PEG, antibodies, and the like or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. A specific example presented herein is the purification of the *Trichinella pseudospiralis* cytokine-like substance using concentration by ultrafiltration and by chromatographic separation on a DEAE anionic column using an LC 100 Chromatography System (Millipore Corp.).

The preferred purification method disclosed herein contains several steps and represents the best mode presently known by the inventors for purification of either TP10 or TP1. This method is currently preferred as it results in the purification, as assessed by immunosuppression for TP10, and stimulation of Th1 cells for TP1, in yields sufficient for further characterization and use. This preferred mode of purification involves the execution of certain purification steps in the order described herein. However, as is generally known in the art, it is understood that the order in which the various purification steps are conducted may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a purified material.

As mentioned above, although preferred for use in certain embodiments, there is no general requirement that the *Trichinella* derived cytokine-like substances always be provided in their most purified state. Indeed, it is contemplated that less purified material, that is nonetheless enriched in their respective activities relative to the natural state, will have utility in
certain embodiments. These include, for example, the production of specific polyclonal and monoclonal antibodies for use in the complete characterization of the Trichinella-derived cytokine-like substances. These immuno-reagents will be useful for the development of kits for use in the rapid identification of different members of the Trichinella sp.. Partially purified fractions for use in such embodiments may be obtained by subjecting a pre-adult worm extract (for TP10) or a newborn extract (for TPi) or supernatant derived by incubating said organisms to one or a combination of the steps described above.

The term "biologically functional equivalent" of TP10 or TPi is used herein to refer to purified substances from nematodes having substantially the same biologic activity of TP10 or TPi as described herein. Thus, from the studies on related organisms presented herein, it can readily be seen that equivalents from other parasites having substantially the same biologic activity are encompassed.

In another aspect, the present invention includes an antibody that is immunoreactive with polypeptide TP10 or TPi. An antibody can be a polyclonal or a monoclonal antibody. In a preferred embodiment, an antibody is a monoclonal antibody. Means for preparing and characterizing antibodies are well known in the art (See, e.g., Antibodies "A Laboratory Manual, E. Howell and D. Lane, Cold Spring Harbor Laboratory, 1988).

Another embodiment of the invention is a method of early prognosis of Trichinella pseudospiralis infection comprising: identifying a human subject suspected of being infected with Trichinella; and assaying a blood sample for the presence of TP10 or TPi.
Yet another embodiment of the present invention is a pharmaceutical composition comprising the TP10 or TPr substance, dispersed in a pharmaceutically acceptable carrier. This substance is useful, as an improved and novel composition for suppressing an immune response comprising treating eukaryotic cells with an effective amount of a pharmaceutical composition; wherein said cells are contacted with the TP10 or TPr substances, or biological equivalents thereof. As used herein the term, "contacted" is used to describe the close juxtaposition of an effective amount of TP10, TPr, or functional equivalents thereof, sufficient to elicit their respective biologic functions.

A related embodiment is a method for altering the immune response of an animal comprising the following steps; preparing a pharmaceutical composition comprising the TP10 or TPr substances dispersed in a pharmacologically acceptable carrier; and administering to an animal a therapeutically effective amount of said substance. A more preferred embodiment is a method for treating an animal comprising; identifying an animal with a need for immunosuppression, administering to an animal a therapeutically effective amount of TP10 or TPr substances dispersed in a pharmaceutically acceptable carrier, wherein the immune response being suppressed is a graft-versus-host response, an autoimmune response, or an allergic response.

As used herein, "pharmacologically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like that do not produce an allergic or similar untoward reaction when administered to a human. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or
agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The preparation of an aqueous composition that contains a protein or proteoglycan as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

A proteoglycan can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like.

For parenteral or intravenous administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent
first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington’s Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

TPi and TP10 have very specific effects on the host’s immune response, that is, they do not appear to exhibit all the broad effects attributed to their respective mammalian counterparts. For TP10 the mammalian counterpart being IL-10; and for TPi the mammalian counterpart being the secretion of mammalian Th1 cytokines gamma interferon and IL-2. The low morbidity and mortality experienced by animals infected with T. pseudospiralis suggests that TP10 and TPi in the host do not induce the side-effects seen in clinical treatment of patients with IL-10 and Th1 cytokine therapy. The specific nature of action for these two molecules would also be of advantage in their application in immunological research. TP10 may be applied in any in vivo or in vitro system to suppress the activation and development of the Th1 subset of T cells (cellular immune responses in general and inflammation specifically). TPi, on the other hand, is a potent inducer of Th1-activating substance by cells capable of manufacturing this material. Release of Th1 cytokines induces T cell
activation, a required initial step in mounting an immune response. Thus, TPi can be used to boost activation of T cells which orchestrate host immunological reaction to non-self materials. The anti-inflammatory effects of TPi come into play when the extended duration of Th1 cytokine release reaches a critical point, whereby the host considers its immune response to be "out-of-control" and some of these cytokines assume their role as communication molecule between the immune system and the HPA axis. At this time they begins to feed back on the HPA axis and increase release of corticosteroids which suppress the runaway immune reaction.

Based on present disclosure it is clear that exudates and extracts of Trichinella pseudospiralis contain materials of great value in both immunology and clinical immunotherapy.

The present invention includes a showing that T. spiralis (all isolates tested) may release materials with activity similar to TP10. The muscle stage of all forms of T. spiralis are enclosed within a collagenous capsule with protects them from immunological destruction by the host immune response. Thus, they do not require so much of a systemic suppression of inflammatory response for their survival as does the unencapsulated muscle stage of T. pseudospiralis. However, plasma corticosterone levels in mice infected with this parasite are elevated, albeit at a much lower level than that seen in mice infected with T. pseudospiralis. These findings indicate that the isolates of T. spiralis may contain a TPi-like molecule. In addition, recent studies indicate that crude antigen from the intestinal parasitic protozoan Cryptosporidium parvum stimulates proliferation of the Th1 helper cell subset in vitro.
A Th2 dominated enteric response is characteristic of infection in mammals with intestinal nematodes in general. Thus, it is possible that the survival strategy employed by T. pseudospiralis to deal with enteritis (TP10) may be employed by other intestinal nematodes as well. Recent studies have revealed that Schistosoma mansoni eggs and the protozoan Trypanosoma cruzi express an oligosaccharide (lacto-N-fucopentaose; LNFIII) that strongly stimulates B cells to proliferate and release IL-10, inhibiting Th1 responses in animals infected with either parasite.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Effects of T. pseudospiralis (TP) infection on the inflammatory response to a heterologous antigen. Inflammatory responses to a one cm piece of sterile cotton string is presented as "Inflammation" measured in terms of units of enzyme activity per mg tissue protein in the granuloma removed from uninfected animals (UI) and TP-infected animals (TP). n=10. UI differed significantly from TP, P<0.05.

FIG. 2. Trinitrochlorobenzene sensitization. Data presented in this FIG. are in terms of activity of a granulocyte marker enzyme in homogenate of ears from uninfected animals (UI) and from animals infected with TP (TP). n=5. UI differed significantly from TP, P<0.05.
FIG. 3. NK tumoricidal effect on P91 tumor cells (10^6) injected subcutaneously. Footpad swelling was measured at 48 hr post challenge. UI differed significantly from TP, P<0.05.

FIG. 4. Comparison of the antiinflammatory effect of a corticosteroid with that of coinfection with TP on myositis in hosts infected with T. spiralis (TS). Inflammation was measured (activity of granulocyte marker enzyme) in the tissue occupied by TS in each of the groups alluded to above. n=10. TS differed significantly from TP + TS and from 0.5 mg CORT, P<0.05. TP + TS and 0.5 mg CORT were similar.

FIG. 5. Establishment of muscle larvae of TS in a host (the Chinese hamster) showing strong resistance to TS in animals infected with either TS alone or with TS and TP (TP + TS). The number of TS becoming established in the tissues of this host was determined. n=10. TS differed significantly from TP + TS, P<0.05.

FIG. 6. Inflammation as determined by granulocyte marker enzyme activity in the tissue occupied by TS in the resistant host was measured for animals infected with TS alone (TS) or concurrently infected with TP and TS (TP + TS). n=5. TS differed significantly from TP + TS P<0.05. Histological analyses were also performed and confirmed the chemical data; not shown).

FIG. 7. Oxidative burst capacity for zymosan-stimulated neutrophils in terms of oxidation of cytochrome c (μMoles cyt c reduced/million cells/hr) in enriched neutrophil preparations collected from uninfected or TP-infected mice by conventional methods. n=7. UI differed significantly from TP, P<0.05.
FIG. 8. Oxidative burst capacity of eosinophils was assessed as in FIG. 7. n=5. UI differed significantly from TP, \( P<0.05 \). Similarly enriched eosinophil preparations were recovered from uninfected or TP-infected mice by conventional methods.

FIG. 9. Peroxidase activity measured from enriched eosinophil preparations. A specific inhibitor of eosinophil peroxidase (3-amino-1,2,4-triazole) was used in controls to measure only eosinophil-specific peroxidase (ESPO). Change in absorbance at 492 nm was recorded every 6 sec for 3 min. Data are presented as Vmax (Mean OD/min). ESPO was measured for eosinophils recovered from uninfected (UI) and TP-infected animals. n=5. UI differed significantly from TP.

FIG. 10. Spleen cells recovered from uninfected (UI) or TP-infected (TP; during depression of inflammation) animals were stimulated with Con A in vitro and the cytokines released were measured. Spleen cells from TP-infected animals released significantly lower amounts of IL-2, \( \gamma \)IFN, and IL-5 than cells from uninfected animals; \( P<0.05 \). n=3.

FIG. 11. Neutrophil chemotactic responses to zymosan-activated serum in the presence or absence of crude extract from TP preadults (500\( \mu \)g/ml). Migration of neutrophils across a membrane with 3-\( \mu \)m diameter pores mounted in a blind well apparatus was determined (% cells crossing membrane) in the absence (ZYM) or presence (Z + Ag) of parasite Ag. n=10. ZYM differed significantly from Z + Ag.

FIG. 12. Oxidative burst (OB) of neutrophils in the presence of no parasite antigen (NO Ag), preadult TP crude Ag at 50, 250, 500 or 750 \( \mu \)g/ml, or 750 \( \mu \)g (750C) of bovine serum albumin (protein control). n=5. All
values obtained for neutrophils incubated in the presence of parasite Ag differed significantly from each other and from both control values. The two control values (NO Ag and 750C) were similar. P<0.05. A strong dose response for effect of parasite Ag on neutrophil oxidative burst was evident.

FIG. 13. The effects of TP preadult ES on the amounts of IL-2, interferon gamma, IL-4, IL-5 and IL-10 released by spleen cells recovered from uninfected mice (UI) and incubated in the presence of either Con A or TP preadult ES. Significantly lower levels of IL-2, interferon gamma and IL-5 were released by spleen cells from UI mice stimulated with TP preadult ES than by those primed with Con A. Data from 3 separate studies in each of which 5 replicates of cells were tested. P<0.05.

FIG. 14. Mesenteric lymph node cells were recovered from uninfected mice (UI) and incubated in the presence of Con A or TP preadult ES and the amounts of IL-2, interferon gamma, IL-4, IL-10 and IL-5 released were measured. The amount of IL-2 released by TP ES-stimulated cells was drastically reduced compared to Con A primed cells (P<0.05). The concentrations of the other cytokines was similar. Data from 3 separate studies in each of which 5 replicates of cells were run.

FIG. 15. Enteritis (inflammation of the small intestine in terms of myeloperoxidase activity) was measured on day 9 PI from mice infected with T. spiralis and injected each day for the first 9 days following infection with either TP preadult (TP 10; PAD ES) ES, medium only (MEDIJC) or remained uninjected (UIJC). (n=12). PAD ES-injected mice showed a dramatic reduction in enteritis when compared to uninjected control mice or medium-injected mice. P<0.05.
FIG. 16. Competitive inhibition of IL-10 detection by antisera from T. pseudospiralis-infected mice. Mean percent inhibition of detection of murine rIL-10 at 300, 100, 33, 11, 3.3 and 1.1 units rIL-10/ml by serum from mice infected with T. pseudospiralis compared to detection in the presence of normal mouse serum. Inhibition was statistically significant at all rIL-10 concentrations tested (P<0.001).

FIG. 17. IL-2 secretion by mesenteric lymph node and spleen cells recovered from TP-infected mice on different days postinfection and stimulated in vitro with Con A. Each bar represents the mean of data from 4 separate studies in each of which 4 replicates of cell cultures were analyzed. IL-2 secretion on day 10 postinfection was significantly greater than that for controls and for days 7, 17 and 24 postinfection (P<0.01).

FIG. 18. Hypersecretion of IL-2 detected in T. pseudospiralis-infected muscle by in situ hybridization. (A) Normal mouse diaphragm muscle and (B) Diaphragm muscle from a mouse following infection with T. pseudospiralis (50 days). Note the strong reaction in connective tissue elements of infected muscle and the absence of such reaction in similar locations within uninfected muscle. (Inset B) T. pseudospiralis larvae (L) do not express IL-2 mRNA above background. Bar = 110μM.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Generally, the nematode parasites of mammals stimulate dramatic inflammatory cell mobilization and recruitment to sites occupied by the parasite. However, in the case of T. pseudospiralis the inflammatory response is almost completely abrogated. Ameliorated
immune responses may be explained by a lack of parasite antigen immunogenicity, masking of the parasite's antigens, antigenic mimicry, or suppression of host cellular responses.

The intestinal phase of infection with *T. pseudospiralis* is only the first of three phases in the parasite's life cycle to which the host must respond. The second stage begins around day 6 postinfection when newborn larvae are shed by adult female worms in the small intestine where they penetrate the lamina propria, invade neighboring tissues, and enter the lymphatics and vasculature. The migration of newborn larvae coincides with a profound suppression of DTH, granuloma formation and contact hypersensitivity responses. The dramatic elevation in plasma corticosterone, which accompanies this down-regulation of cellular immunity in the *T. pseudospiralis*-infected host, is believed to be due to either the elaboration of parasite-derived factor(s) which act directly on the pituitary or adrenals and thereby induce corticosteroid release, or to the larvae stimulating the release of neuroendocrine-active cytokines by host cells. Since parasite ES have no effect on ACTH release by pituitary cells or on corticosteroid release by adrenal cells in vitro (Stewart, unpublished), the *T. pseudospiralis*-infected host was examined for perturbations in T cell cytokine release.

Studies of the closely related *T. spiralis*, have extensively documented the cellular immune response to *Trichinella* infection. The susceptibility of mice to *T. spiralis* has been determined for a number of Major Histocompatibility (MHC) antigen backgrounds, and correlates with the differential activation of the helper T cell subset type that directs the immune response. Susceptibility of inbred hosts correlates with T helper-2
(Th2) driven immune responses, as characterized by the secretion of interleukins-3, -4, -5, -9, and -10. The primary functions of IL-10 are to antagonize the effects of T helper-1 (Th1) cells, and promote Th2 driven B cell help. T. spiralis is a strong inducer of the Th2 phenotype by, for example, inducing the secretion of IL-10 by T cells, thereby skewing the immune response toward the susceptible phenotype.

The present application discloses novel substances that have immunomodulatory capabilities and are isolatable from parasitic nematodes, such as Trichinella pseudospiralis. All parasites of vertebrates must evade host immune response in order to survive. However, by virtue of the site it occupies in the host, this parasite should elicit, as do other closely related parasites, a violent immune response by the host. Thus, T. pseudospiralis must employ far-reaching and effective immunoevasive strategies to insure its survival. Its most pressing requirement in this regard is to evade host inflammatory response, an obstacle which the host presents to every stage in the parasite's life cycle.

The inventor has identified and isolated the parasite components responsible for such evasion and has assessed the applicability of these components in the control of vertebrate inflammatory response, both specifically and systemically. The present invention takes advantage of the newly discovered substances that the parasite uses to control the host inflammatory response. The novel substances of the present invention are, for example, widely applicable to a wide variety of medically important autoimmune diseases seen in humans. This applicability is based on the strong anti-inflammatory, and immunomodulatory effects exerted by TP10 and TP1.
The present invention discloses the elaboration of two novel parasite-derived components that directly and indirectly regulate T cell responses in murine hosts infected with *T. pseudospiralis*. One component, TP10, is secreted or exuded by the preadult stage of *T. pseudospiralis* and displays IL-10-like properties.

A second component (TPi) is produced by newborn larvae and induces hypersecretion by host lymphoid and non-lymphoid cells of a potent cytokine-like substance that dramatically stimulates cells of the Th1 subset of lymphocytes to hypersecrete their characteristic profile of cytokines, including IL-2, interferon gamma and IL-3, resulting in downregulation in systemic cellular immunity. The secretion/excretion of these two molecules coincides with specific stages in the parasite's life history when the worms are at greatest potential risk to immunological destruction.

The *T. pseudospiralis* nematode is parasitic in a wide variety of mammals (including several species of commonly employed laboratory animals) in which it invades deep tissues and survives for the life of the host. The present inventor characterized the surprising lack of inflammation in response to tissue invasion by this parasite (Stewart et al., 1985). This paucity of host cellular response has been documented both histologically, and chemically by assaying for inflammatory cell marker enzymes. Despite the parasite's extensive migratory activities within a variety of host tissues, there is little morbidity or mortality associated with even massive infections (Stewart, 1989).

TP10 is excreted by the preadult worm stage in the life cycle of the parasite *T. pseudospiralis*. TP10 is very similar to native mammalian interleukin 10 (IL-10) in terms of its ability to shut down a Th1 response (Th1
cells promote and orchestrate inflammatory cellular immunity). TP10 may be used in the same clinical settings that IL-10 is used, as is apparent, for example, from the in vivo examples disclosed herein.

Several characteristics of TP10 render it of greater value than IL-10 in such applications. The wide range of uses for which IL-10 has been employed in testing and commercial enterprises have demonstrated a wide variety of undesired side effects IL-10 elicits. This is not the case with TP10, since the only effect of TP10 on host immunity noted thus far is the anti-Th1 effects.

The millions of years during which this parasite has had to adapt to the host immune response have allowed the parasite to fine-tune the action of TP10 so that it is very specific in its action, altering only those immune elements that endanger the parasite’s survival. Thus, TP10 is very specific in its effects, shutting down the Th1 response in a highly effective manner, while not impacting on unrelated aspects of host immunological responsiveness. In this sense, TP10 enjoys an advantage over native IL-10, which has broader effects including some undesired or harmful effects on host immunity. Some of the applications for TP10 are:

a. By mimicking IL-10 it suppresses cell-mediated immunity, supporting its usefulness in a wide variety of autoimmune diseases;

b. Suppression of a Th1 response to stop production of the cytokine mediators of septic shock (primarily IL-1 and TNF), allowing it to be applied in treating life threatening conditions;
c. IL-10 is also used routinely in the basic research and biomedical research laboratories. TP10 is useful for similar applications and for use as a therapeutic agent.

At another stage in the life cycle of *T. pseudospiralis*, the newborn larvae (NBL), secretes/excretes a substance (TPi) that is a powerful inducer of release by vertebrate lymphoid and non-lymphoid cells of a cytokine-like substance which stimulates Th1 cells to proliferate and release copious amounts of their characteristic cytokines. In the host the activity of this parasite-derived substance (TPi) can be measured in terms of IL-2 or interferon gamma levels (cytokines that are elaborated by activated Th1 cells). In vitro the activity of TPi can be measured in terms of its ability to stimulate fibroblasts and T cells to release a cytokine-like substance which caused proliferation of HT2 cells, an IL-2 dependent cell line, or by activation and proliferation of Th1 cells. The ability of this parasite-induced molecule to stimulate HT2 cell proliferation suggests that it may be similar in some ways to IL-2. When applied in an HT2 proliferation assay, the TPi-induced molecule released by T cells or fibroblasts showed an HT2 proliferation response as much as 20,000 times greater than controls. When fibroblasts were exposed in vitro to live newborn larvae of *T. pseudospiralis*, these cells released material that induced HT2 proliferation at levels 500,000 times that seen with controls. The potency of TPi in inducing release of this IL-2-like activity by host cells is no less than phenomenal.

**Additional characterization of TPi**

In an effort to identify the IL-2-like substance which 3T3 cells release under the influence of TPi, the
following studies have been conducted and have provided the following characteristics and results:

a. cDNA was recovered from 3T3 cells following 48 hr exposure to TPi and then used in a polymerase chain reaction for IL-4, IL-6, IL-7, GM-CSF, gamma interferon, IL-12, and IL-2. Results were negative for all cytokines tested.

b. Supernatant from 3T3 cell cultures stimulated with TPi for 48 hr were run in the HT2 assay. An attempt was made to block HT2 proliferation with anti-IL-2, anti-IL4, anti-IL-12 and anti-IL-7 antibodies. None of these antibodies inhibited HT2 proliferation.

c. RNA was purified from preadult T. pseudospiralis and run in the same PCR as in "a" above. There was no positive reactions in the PCR for the cytokines tested.

d. Preadult ES was fractionated by application to a liquid chromatography system (LC100, Millipore Corp.) using an anionic DEAE column. Elution was at (pH 8) with a gradual increase in concentration of NaCl over a 30 min period from 10% to 100% of a 1M NaCl solution. A total of 10 major peaks were eluted from the column. Preadult ES fractions were tested for their ability to inhibit cytokine (IL-2, IL-3 and interferon gamma) release by HDK-1 cells. Dramatic reductions in all three cytokines below controls were seen with 4 out of the 10 peaks.

The TPi of the present invention has been used by the present inventor in routine research to greatly boost natural killer cell activity in vivo and to promote growth of IL-2-dependent cell lines. Hence, it is apparent that TPi has numerous applications in basic and biomedical research settings in immunology.
TPi will also be useful in clinical settings. TPi should be especially helpful in the treatment of patients with certain immunocompromising conditions such as AIDS, radiation-induced or chemotherapy-induced suppression of host immunity, some immunosuppressive forms of cancer, and the like. Again, the strong specificity of activity associated with these parasite-derived substances will limit any diverse effects usually seen with therapy employing native cytokines which often have a broad range of activities, some of which are detrimental.

The present invention also concerns the effect of TPi on a model for T cell-mediated immune response in vivo. The enteric response of the host to infection with a parasite closely related to T. pseudospiralis was dramatically augmented in mice injected with TPi. This demonstrated, along with augmentation of in vivo natural killer cell activity, that this substance directly influences IL-2-inducible immune responses in the intact vertebrate host.

The present disclosure shows that T. pseudospiralis employs several different immunoaevasive strategies to insure its survival. Initial efforts related to the specific mechanism by which the parasite successfully infects a host included analyses of the functional status of specific cell types assigned to play an effector role in nematode infections. To quantitate the effects of T. pseudospiralis ES products on specific cell populations, the oxidative burst in neutrophils and eosinophils was determined and found to be suppressed during infection with this parasite. Likewise, eosinophil peroxidase levels were greatly affected.

The quantities of key cytokines released by Con A-stimulated spleen cells from animals infected with T. pseudospiralis are profoundly different from those
seen with cells from uninfected animals. These results demonstrate that a parasite-induced modulation of T cell subset activation during infection was potentially induced by this parasite. Gross changes in inflammatory response (as determined histologically) and the above described perturbations in neutrophil and eosinophil function are causally related to parasite modulation of T cell subset activation.

The two currently recognized T cell subsets that would be expected to play a role in the host’s response to infection have been designated Th1 and Th2. Th1 cells are known to up-regulate inflammatory response. The parasite could manipulate inflammatory response by preventing the activation of Th1 cells. If the parasite were able to control T cell activation, it could accomplish this in a number of ways. Nematodes exude a wide variety of substances which are collectively referred to as excretory/secretory products (ES). The ES of T. pseudospiralis are strong candidates for participation in this parasite’s ability to modulate host response since crude extracts from the parasite dramatically suppress chemotactic response and oxidative burst in neutrophils from uninfected animals. In addition, this same material alters the pattern of cytokine release by spleen and lymph node cells from uninfected animals.

A finding of the present invention is also that the closely related parasite Trichinella spiralis may also elaborate substances with similar effects to those shown for TP10 and TP1, albeit at apparently much lower levels that T. pseudospiralis.
Biologically Functional Equivalents

As mentioned above, modification and changes may be made in the structure of the Trichinella TP10 and TPi and still obtain a substance having like or otherwise desirable characteristics. For example, insofar as the substance is substantially proteinaceous, certain amino acids may be substituted for other amino acids in protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a protein with like or even countervailing properties (e.g., antagonistic v. agonistic). Thus various changes may be made in the sequence of the Trichinella cytokine-like protein substances or peptides without appreciable loss of their biological utility or activity.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte & Doolittle, J. Mol. Biol., 157:105-132, 1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are:

- isoleucine (+4.5); valine (+4.2); leucine (+3.8);
- phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7);
serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydrophobic character of the amino acid determines the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, cytokines, antibodies, antigens, and the like. It is known in the art that an amino acid may be substituted by another amino acid having a similar hydrophobic index and still obtain a biological functionally equivalent protein. In such changes, the substitution of amino acids whose hydrophobic indices are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biological functional equivalent protein or peptide thereby created is intended for use in immunological embodiments. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e. with a biological property of the protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5);
leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

While discussion has focused on functionally equivalent polypeptides arising from amino acid changes, it will be appreciated that these changes may be effected by alteration of the encoding DNA; taking into consideration also that the genetic code is degenerate and that two or more codons may code for the same amino acid.

Epitopic Core Regions

U.S. Patent 4,554,101 (Hopp, incorporated herein by reference) also teaches the identification and preparation of epitopes from primary amino acid sequences on the basis of hydrophilicity. Through the methods disclosed in Hopp one of skill in the art would be able
to identify epitopes from within an amino acid sequence of the TP10 and TP1 protein sequence(s). These regions are also referred to as "epitopic core regions".

Numerous scientific publications have been devoted to the prediction of secondary structure, and to the identification of epitopes, from analyses of amino acid sequences (Chou-Fasman, 1974a,b; 1987a,b; 1979). Any of these may be used, if desired, to supplement the teachings of Hopp in U.S. Patent 4,554,101. Moreover, computer programs are currently available to assist with predicting antigenic portions and epitopic core regions of proteins. Examples include those programs based upon the Jameson-Wolf analysis (Jameson & Wolf Compu. Appl. Biosci., 4(1):181-6, 1988; Wolf et al. Compu. Appl. Biosci., 4(1):187-91 1988). More recently, new programs have become available for use in predicting protein tertiary structure (Fetrow & Bryant, 1993), which may also be taken into consideration when analyzing likely epitopic core regions using values such as those presented therein.

ANTIBODIES

In another aspect, the present invention contemplates an antibody that is immunoreactive with a component of the invention. An antibody can be a polyclonal or a monoclonal antibody. In a preferred embodiment, such an antibody is a monoclonal antibody. Means for preparing and characterizing antibodies are well known in the art (See, e.g., Antibodies "A Laboratory Manual, E. Howell and D. Lane, Cold Spring Harbor Laboratory, 1988).

Briefly, a polyclonal antibody is prepared by immunizing an animal with an active or deactivated immunogen comprising a component of the present invention.
and collecting antisera from that immunized animal. A wide range of animal species can be used for the production of antisera as is known to those of skill in that art. Typically, but not exclusively, an animal used for production of anti-antisera is a goat, rabbit, mouse, rat, hamster or guinea pig. Because of the relatively large blood volume of rabbits and goats, a rabbit or a goat is a preferred choice for production of polyclonal antibodies.

Antibodies, both polyclonal and monoclonal, specific for the Trichinella derived substance or component of the present invention may be prepared using conventional immunization techniques, as will be generally known to those of skill in the art. A composition containing antigenic epitopes of the Trichinella substances can be used to immunize one or more animals, such as a rabbit or mouse, which will then proceed to produce specific antibodies against TP10 or TP1 as the case may be. Polyclonal antisera may be obtained, after allowing time for antibody generation, simply by bleeding the animal and preparing serum samples from the whole blood.

To obtain monoclonal antibodies, one would also initially immunize an animal, often preferably a mouse, with an immunogenic composition comprising either TP10 or TP1. One would then, after a period of time sufficient to allow antibody generation, obtain a population of spleen or lymph cells from the animal. The spleen or lymph cells can then be fused with cell lines, such as human or mouse myeloma strains, to produce antibody-secreting hybridomas. These hybridomas may be isolated to obtain individual clones which can then be screened for production of antibody to the desired substance having cytokine-like activity.
Following immunization, spleen cells are removed and fused, using a standard fusion protocol (see, e.g., The Cold Spring Harbor Manual for Hybridoma Development, incorporated herein by reference) with plasmacytoma cells to produce hybridomas secreting monoclonal antibodies against the respective substance. Hybridomas which produce monoclonal antibodies to the selected antigens are identified using standard techniques, such as ELISA and Western blot methods.

Hybridoma clones can then be cultured in liquid media and the culture supernatants purified to provide the TP10 or TPI-specific monoclonal antibodies. In general, monoclonal antibodies to the desired TP10 or TPI antigen of Trichinella can be used in both the diagnosis and treatment of Trichinosis infections.

It is proposed that the monoclonal antibodies of the present invention will find useful application in standard immunochemical procedures, such as ELISA and Western blot methods, as well as other procedures which may utilize antibody specific to antigenic Trichinella cytokine-like substance epitopes. These specific monoclonal antibodies are anticipated to be useful in various ways for the treatment of Trichinella infections through, for example, their application in passive immunization procedures.

Additionally, it is proposed that monoclonal antibodies specific to these particular Trichinella derived substances may be utilized in other useful applications. For example, their use in immunoabsorbent protocols may be useful in purifying native or recombinant TP10 or TPI species or variants thereof.

In general, both poly- and monoclonal antibodies against TP10 or TPI may be used in a variety of
embodiments. They may be used in inhibition studies to analyze the effects of blocking the actions of the components released during a *Trichinella* infection in cells or animals. Anti-TP10 or -TPi antibodies will also be useful in immunolocalization studies to analyze the distribution of TP10 and TPi during various cellular events, for example, to determine developmental expression during the different stages of the nematodes life cycle, and their correlation with pathogenicity. A particularly useful application of such antibodies is in purifying native or recombinant TP10 or TPi, for example, using by using an antibody affinity column. The operation of all such immunological techniques will be known to those of skill in the art in light of the present disclosure.

**Pharmaceutical Compositions**

The active compounds may also be administered parenterally or intraperitoneally. Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for parenteral use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of
microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial ad antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus ny additional desired ingredient from a previously sterile-filtered solution thereof.

The active substances of the present invention may be orally administered, for example, with an inert
diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with food. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.01% of active compound. The percentage of the compositions and preparations may, of course, be varied. The amount of active substances in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compounds sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the
active compounds may be incorporated into sustained-release preparation and formulations.

TPi and TP10 have very specific effects on the host's immune response, that is, they do not appear to exhibit the broad effects attributed to their mammalian counterparts (IL-2-like activity and IL-10). Furthermore, the low morbidity and mortality experienced by animals infected with the Trichinella pseudospiralis parasite further corroborate that these two materials do not induce the side-effects observed in the clinical treatment of patients with IL-10 and IL-2-like activity.

The very specific nature of action of TPi or TP10 is also of advantage in their application in immunological research. TP10 could be applied in any in vivo or in vitro system in which one wished to suppress the activation and development of the Th1 subset of T cells (cellular immune responses in general and inflammation specifically). TPi is a potent inducer of production/release of IL-2-like activity by cells of the vertebrate capable of manufacturing this cytokine (e.g., fibroblasts and T cells). The release of the IL-2-like molecule follows T cell activation, the required initial step in mounting an immune response. Thus, TPi can be used just like IL-2 to boost activation of T cells which orchestrate host immunological reaction to non-self materials. The anti-inflammatory effects of TPi come into play when the duration of IL-2 release reaches a critical point and the neuro-immunologic feedback mechanism is activated to control IL-2 release. Therefore TPi may play a role as a communication molecule between the immune system and the HPA axis. The feedback response from the HPA axis increases the release of corticosteroids which suppress the runaway immune reaction.
The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1
CHARACTERIZATION OF TRICHINELLA PSEUDOSPIRALIS CYTOKINE-LIKE SUBSTANCES

The identification of novel Trichinella pseudospiralis derived products was surprising, given the previous studies conducted on Trichinella spiralis and other helminth parasites. Delayed-type hypersensitivity responses were assessed to antigens unrelated to the parasite in animals infected with T. pseudospiralis and found that the host response to three different unrelated antigens: cotton string (FIG. 1); tumor cell antigens (FIG. 2); and trinitrochlorobenzene (FIG. 3). These responses were dramatically suppressed (Stewart et al., 1991) in the presence of a T. pseudospiralis infection.

The immunosuppressive capacity of T. pseudospiralis was also examined by comparison with the dynamic interactive effects of immune-associated events due to infection with another parasite. Hosts infected with T. pseudospiralis were challenged with the closely related nematode parasite, Trichinella spiralis, a helminth known to induce dramatic inflammation at several host sites.
(Stewart et al., 1985). Inflammation at all sites that normally exhibit a response to T. spiralis was dramatically suppressed as determined by histochemical and chemical means. The success of T. spiralis, as measured in terms of number of muscle larvae, increased by 57% in hosts concurrently infected with T. pseudospiralis compared to those with T. spiralis alone.

METHODS

1. Heterologous Antigen Assay. The effect of T. pseudospiralis infection of the inflammatory response to a heterologous antigen was measured as follows. A one cm piece of sterile cotton string was implanted subcutaneously in animals infected with T. pseudospiralis (TP). At seven days post-implant, the string and surrounding granuloma tissue were removed and measured for protein content and activity of a marker enzyme for granulocytes. Data presented in FIG. 1 as "Inflammation" are measured in terms of units of enzyme activity per mg tissue protein in the granuloma removed from uninfected animals (UI) and TP-infected animals (TP). n=10. UI had a significantly greater inflammatory response than TP infected animals, P<0.05. Histological analyses confirmed these chemical data (not shown). Inflammation was measured in terms of a marker enzyme for granulocytes (myeloperoxidase). This technique was applied to assess inflammatory response in studies examining enteritis, myositis and granuloma formation. In measuring enteritis, the entire small bowel was removed from the animal, rinsed out with 20 ml of sterile saline using a 20 cc syringe, split lengthwise, rinsed in sterile saline, the entire mucosa was removed and homogenized in 2 ml of sterile saline with 30 strokes of a glass homogenizer on ice. Myeloperoxidase activity and total protein was measured in this homogenate. For measuring inflammation in string granulomas, the implanted string and surrounding granulation tissue were removed from
their subcutaneous location and treated as above for homogenization and measurement of myeloperoxidase activity and total protein. Measurement of myositis or ear inflammation involved removal of the entire diaphragm, pectoralis and/or masseter muscles or ear followed by homogenization (50 strokes) and measurement of myeloperoxidase activity and total protein as above in the homogenate. Myeloperoxidase activity was measured as described by Charniga et al. (1981). The effects of host sex on enteric response to infection with Trichinella spiralis. Briefly, to a cuvette were added 1 ml of 20mM aqueous guaiacol (Sigma Chemical Company, St. Louis, MO), 2.0 ml of 100mM phosphate buffer (pH 6.0), 50-500 µl of mucosal, muscle or granuloma homogenate and 20 µl of 150mM H₂O₂. The increase in absorbance at 470 nm at ambient temperature was determined every 15 sec for 3 min on a spectrophotometer. The relationship between moles of H₂O₂ decomposed and change in optical density was determined by measuring the change in absorbance using increasing amounts of H₂O₂ in the presence of excess standard horseradish peroxidase (Sigma). Protein was measured using the spectrophotometric, coomassie blue protein-binding technique of Sedmak and Grossberg (J.J. Sedmak and S.F. Grossberg (1977)). A rapid, sensitive and versatile assay for protein using Coomassie Brilliant Blue G250. Thus, µMoles of H₂O₂ decomposed/min/mg protein are the units presented in graphs which include an X-axis title of "Inflammation".

2. Trinitrochlorobenzene (TCNB) Contact Sensitization Assay. TNCB in alcohol was applied to the shaved abdomen of animals. Five days later the right ear of each animal was painted with TNCB in olive oil (challenge). Forty-eight hours following challenge, the right ear was removed, homogenized and examined for inflammation histologically (data not shown) and chemically. Data presented in FIG. 2 are measured in terms of activity of
a granulocyte marker enzyme in homogenate of ears from uninfected animals (UI) and from animals infected with TP. n=5. UI inflammation was significantly greater than that from TP infected animals, P<0.05. Controls included measurement of inflammation in the unchallenged left ear of the same animals, in ears from animals not sensitized and not challenged, and in ears from animals either challenged but not sensitized, or sensitized but not challenged. Histological analyses of ears from all groups confirmed chemical data.

3. Tumor Specific DTH Assay. The P91 mastocytoma, a mutagenized derivative of P815 mastocytoma (H-2d), was originally obtained from Dr. T. Boon (Ludwig Cancer Institute, Brussels, Belgium). Tumor cells were originally grown in Dulbecco’s modified Eagle’s minimal essential medium (MEM; GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 1% L-glutamine, 1% vitamin solution, 1% sodium pyruvate 1% streptomycin-penicillin-fungizone (=complete medium). (10^6) were injected subcutaneously in animals uninfected and infected with TP. Fourteen days later these animals were injected in the footpad with P91 tumor antigens and footpad swelling was measured at 48 hr post challenge, as shown in FIG. 3. Controls consisted of animals sensitized but not challenged, challenged but not sensitized and animals challenged and sensitized with vehicle only. UI footpad swelling was significantly greater that from TP infected animals, P<0.05.

RESULTS

Effects of Coinfection on Host Inflammatory Response. Six-8 week old ICR Swiss albino mice (Harlan Sprague-Dawley, Houston, TX), hosts normally permissive to T. spiralis (TS) were infected with TS alone (TS) or concurrently with TS and T. pseudospiralis (TP + TS). Inflammatory response was measured in the diaphragm and
pectoralis muscles of hosts infected with *T. spiralis* (FIG. 4). A third group of similarly measured animals were infected with TS alone but received daily intramuscular (im) injections of 0.5 mg cortisone acetate suspended in 0.1 ml of sterile saline from the day of infection until the completion of the study (0.5 mg CORT). Controls infected with TS received daily injections of sterile saline only and did not differ from TS alone. Inflammation was measured in terms of myeloperoxidase activity in diaphragm muscle from animals in each of the above groups. n=10. Muscle from TS was significantly more inflamed than muscle from TP + TS or 0.5 mg CORT, P<0.05. TP + TS and 0.5 mg CORT were similar. Histological analyses were also performed and confirmed the chemical data.

**Effects of Coinfection on Host Susceptibility.**
Hosts with demonstrated strong resistance to infection with *T. spiralis* (TS) were infected with either TS alone or with TS and TP (TP + TS). The number of TS becoming established in the tissues of this host was determined as shown in FIG. 5. n=10. TS was significantly less infected that TP + TS, P<0.05, thus demonstrating that TP decreased host resistance to TS. Male Chinese hamsters (*Cricetulus griseus*) were 4-5 weeks old when used as hosts (Cytogen Research and Development, West Roxbury, MA). Total numbers of larvae becoming established in the muscles of each treatment group of hosts were determined by digestion of skinned, eviscerated carcasses homogenized in a Waring blender in 250 ml of 1% pepsin/HCl and incubated in a water bath shaker for 3 hr at 37°C. Reproductive capacity index (RCI) was used to express infectivity of parasites. TCI is calculated by dividing the total number of muscle larvae recovered after day 40 postinfection from the entire body musculature of an animal by the number of infective larvae administered to the host during infection. Total
worm numbers were determined by aliquot counting at 10X using a stereoscopic microscope. As larvae recovered from hosts infected with both species of parasite were counted they were assigned to one or the other species based on differences in length and width.

**Effects of Coinfection on Inflammation.** FIG. 6 demonstrates the effects of *Trichinella sp.* coinfection on granulocyte marker enzyme activity (inflammation) in diaphragm and pectoralis muscle from Chinese hamsters (a host normally resistant to infection with *T. spiralis*) infected with *T. spiralis* alone (TS) or concurrently infected with *T. spiralis* and *T. pseudospiralis* (TP + TS). Muscle from hamsters infected with TS alone were significantly more inflamed than muscle infected with both TS and TP. n=5. P<0.05. Histological analyses were also performed and confirmed the chemical assay.

**Effect of TP on Oxidative Burst.** FIG. 7 demonstrates the effects of TP infection on oxidative burst as measured from enriched neutrophil preparations. Neutrophil preparations were collected by conventional methods from the peritoneal spaces of uninfected (UI) or TP-infected animals (TP) 12 hr following intraperitoneal injection with 2 ml of 0.2% glycogen in sterile saline. Oxidative burst capacity was assessed for zymosan-stimulated neutrophils in terms of oxidation of cytochrome c (μMoles reduced cyt c/million cells/hr). n=7. UI showed significantly more oxidation that TP, P<0.05. Similarly eosinophil preparations were recovered by conventional methods from the peritoneal spaces of uninfected (UI) or TP-infected (TP) animals 48 hr following ip injection of 0.2% glycogen in sterile saline. As shown in FIG. 8, oxidative burst capacity of eosinophils was assessed as in FIG. 7. n=5. UI showed significantly more oxidation than TP, P<0.05. Neutrophils or eosinophils (5 x 10^5) in 1 ml of Krebs-
ringer phosphate buffer were preincubated for 5 min at 37ºC in polypropylene tubes. Negative controls consisted of incubates as above with 30 µg of superoxide dismutase (Sigma) added to inhibit superoxide anion-dependent reactions. The reaction was started by the addition of 0.1 ml of 1.2 mM ferricytochrome c (Sigma) and 0.1 ml of opsonized zymosan at 10 mg/ml. Opsonized zymosan was prepared by suspending zymosan (Sigma) in sterile saline at concentration of 50mg/ml with incubation of 1 volume of this preparation with 4 volumes of fresh, nonheat-inactivated, mouse serum from uninfected mice for 30 min at 37ºC. After incubation, zymosan was collected by centrifugation and resuspended in sterile saline at 10 mg/ml for use in these studies. Following activation of granulocytes by exposure to opsonized zymosan for 10 min at 37ºC, the reaction was stopped by placing the tubes containing granulocytes in an ice bath, and promptly centrifuging the tubes at 400 x g at 4ºC for 10 min. Absorbance of triplicate samples of the supernatants of these granulocyte suspensions at 550 nm was determined in an Array 3000 Spectrophotometer. Oxidative burst is presented in terms of micromoles of cytochrome c reduced/million cells per hr.

Specific Eosinophil Peroxidase Activity. In FIG. 9, peroxidase activity was measured from enriched eosinophil preparations. A specific inhibitor of eosinophil peroxidase (3-amino-1,2,4-triazole) was used in controls to measure only eosinophil-specific peroxidase (ESPO). The assay is based on the oxidation of o-phenylenediamine by ESPO in the presence of H₂O₂. Eosinophils were activated by incubation in the presence of cytochalasin B and PMA prior to measurement of ESPO. Change in absorbance at 492 nm was recorded every 6 sec for 3 min. Data are presented as Vmax (Mean OD/min). ESPO was measured for eosinophils recovered from uninfected (UI)
and TP-infected animals. n=5. UI peroxidase activity was significantly greater than that from TP.

**Spleen Cell Activation.** Splenocytes were recovered under sterile conditions by conventional methods from uninfected or on day 17 postinfection from *T. pseudospiralis*-infected (TP) 6-8 wk old female C57/BL6 mice (Jackson Labs, Bar Harbor, ME) and stimulated during 24 hr incubations at 37°C under 5% CO$_2$ with Con A (5 μg/well of 96-well plates containing 10$^6$ splenocytes/well) and supernatants were analyzed for IL-2 (using the HT2 IL-2 dependent cell proliferation assay), gamma interferon, IL-4, IL-5 and IL-10 (measured by ELISA). Spleen cells from TP-infected animals released significantly lower levels of IL-2, interferon gamma and IL-5 at this point during infection when high levels of plasma corticosterone are found in the TP-infected host (Stewart et al., 1988) than did cells from uninfected animals, as shown in FIG. 10; P,0.05, n=3.

**Chemotaxis Assay.** Enriched neutrophil preparations recovered from uninfected animals by methods outlined above were tested for chemotactic response to zymosan-activated serum in the presence or absence of crude extract from TP preadults (500μg/ml). FIG. 11 shows the migration of neutrophils across a membrane with 3-μm diameter pores mounted in a blind well apparatus and cell numbers determined (% cells crossing membrane) in the absence (ZYM) or presence of parasite Ag (Z + Ag). n=10. ZYM showed significantly more migration that Z + Ag. Neutrophil chemotaxis was assessed using enriched neutrophil preparations recovered from the peritoneum of uninfected mice 9 hr following intraperitoneal injection of 2 ml of 0.2% oyster glycogen (Sigma) suspended in RPMI 1640 containing 20% normal mouse serum and 2% antibiotic/antimycotic (streptomycin [10,000 μg/ml]- penicillin [10,000 units/ml]-fungizone [amphotericin B, 25
µg/ml); GIBCO). The bottom well of the blind well chamber (Nucleopore Corp., Pleasanton, CA) was filled with 0.2 ml of a solution containing the solution to be tested for chemotactic potential. A membrane with 3 µm pores was placed over the bottom chamber of the well. The plastic top chamber was screwed in place over the membrane to form the upper chamber of the well. The top chamber was filled with 0.8 ml of neutrophil suspension (10^6 cells/0.8 ml). Blind wells were incubated at 37°C under 5% CO₂ for 90 min. following incubation, the top chamber was removed and the membrane fixed in methanol and stained in 0.3% aqueous Toluidine Blue for 1.5 min, rinsed in deionized water and placed on a glass slide to air dry over night. The following day the membranes were mounted bottom-side up on a glass slide with permount (Sigma) and an ocular grid mounted in a compound microscope was used to determine the number of neutrophils at 40X in 10 random grids migrating completely through the membrane to the other side was determined. Chemotactic response of neutrophils to the potent chemotactic agent, zymosan-activated normal, non-heat inactivated mouse serum in the presence or absence of TP preadult ES. Since neutrophils participate in inflammatory responses, and since inflammation is a host response that the preadult TP must evade until they have completed reproduction in the host bowel, the presence of a molecule in the ES products of TP preadults which overcomes the powerful neutrophilotactic qualities of zymosan-activated mouse serum and repels neutrophils fits teleologically with the overall immunoevasive strategy of this parasite.

Oxidative Burst. Oxidative burst (OB) was measured in enriched neutrophil preparations recovered form uninfected animals and incubated in the absence of parasite antigen (NO Ag), in the presence of preadult T. pseudospiralis ES (50, 250, 500 05 750 µg/ml) or in the
presence of a protein control consisting of 750 μg/ml bovine serum albumin (750C). All values obtained for neutrophils incubated in the presence of parasite Ag were significantly less than either control and also differed from each other (FIG. 12). n=5 P<0.05. The two control values were similar. A strong dose response for effect of parasite Ag on neutrophil oxidative burst was evident. These data agree with those presented in FIG. 11 above, reflecting parasite induced perturbations in neutrophil function which may hinge on the ability of these parasite products to modulate cytokine expression by T cells (some T cells contaminate any preparation of neutrophils recovered from animals) which in turn impinges on neutrophil function.

Among the first indications that T. pseudospiralis (TP) preadult ES might directly impact cytokine release by T cells came from studies in which spleen cells (FIG. 13) and mesenteric lymph node cells (MLN; FIG. 14) were recovered by conventional methods from uninfected 6-8 wk-old, female C57/BL6 mice (Jackson Labs) and exposed form 24 hr in vitro to either Con A or to TP preadult ES. Following incubation the amounts of the cytokines IL-2, gamma interferon, IL-4, IL-5 and IL-10 were measured in culture supernatants. Data were obtained from 3 separate studies with 5 replicates for each group. Analysis of spleen cell culture supernatants revealed that exposure to TP preadult ES was accompanied by release of significantly lower levels of IL-2, gamma interferon, and IL-5 than was evident in cultures exposed to Con A. P<0.05. The levels of all other cytokines examined were similar between groups. Supernatants from MLN cultures exposed to parasite antigen showed a dramatic and significant suppression of IL-2 release compared to cells incubated in the presence of Con A. P<0.05. The concentrations of all other cytokines examined were similar between the two groups. IL-2 was measured using
the HT2 IL-2 dependent proliferation assay. Other cytokines were measured using cytokine specific ELISAs.

Mice infected with T. spiralis remained uninjected (UINJ) or were injected each day for the first 9 days following infection with either partially purified TP preadult ES (TP10) (designated as PAD ES), medium only (MEDIJC). Enteritis (inflammation of the small intestine was measured in terms of myeloperoxidase activity) on day 9 post-infection in all mice from each group (n=12) was determined. FIG. 15 demonstrates that TP10-injected mice showed a dramatic reduction in enteritis when compared to uninjected control mice or medium-injected mice. P<0.05.

**Competition Assay Using TP infected Antisera.**

Although TP10 displayed some of the activities of mammalian IL-10, the parasite molecule was undetectable by a standard ELISA for IL-10. However, when serum from mice infected with T. pseudospiralis, which should contain antibodies against TP10, was tested for its ability to bind to rIL-10 and inhibit its detection by an ELISA specific for IL-10, some cross-reactivity between TP10 and IL-10 was demonstrated. Mean percent inhibition by 100 μl of serum from mice infected for 45 days with T. pseudospiralis of detection of murine rIL-10 (300, 100, 33, 11, 3.3 and 1.1 units rIL-10/ml) using an IL-10 specific ELISA (FIG. 16). While normal mouse serum controls did not inhibit IL-10 detection, serum from infected mice inhibited IL-10 detection to a statistically significant degree at all concentrations tested (P<0.001). All serum samples were heat inactivated. n=5.

TP10 elaborated by intestinal stage of T. pseudospiralis inhibits enteritis. Intestinal inflammation on day 9 postinfection measured as mean myeloperoxidase activity ±S.D. (μMoles H₂O₂
decomposed/min/mg protein) in the small intestines of 6-8 wk-old female ICR Swiss albino mice infected with T. spiralis. Mice received daily intraperitoneal injection of T. pseudospiralis preadult ES (n=10), medium alone (n=4) or no injection (n=5) beginning 2 days before infection continuing through the ninth day postinfection. Myeloperoxidase activity in the small intestines of mice injected with preadult ES differed significantly (P<0.05) from either control group.

The ES of stage 2 in the life cycle of the parasite (newborn larvae) are referred to hereafter as NBL ES. Those of stage 4, (preadult worms) are referred to as PAD ES. Studies on PAD ES now show that they contain a molecule, TP10, that mimics in activity the mammalian cytokine, IL-10. This is an exciting and heretofore unreported finding. IL-10 is a substance released by mammalian T cells that, among a broad range of other functions, controls the development of the T cell subset destined to orchestrate the entire immune response the host will mount, suppressing the activation of Th1 cells (the T cell subset that promotes cellular response) and promoting the development of Th2 cells. Mice infected with T. spiralis were injected ip with PAD ES to see if TP 10 would alter host response to a different parasite. Intestinal inflammations in mice injected with medium alone or PAD ES were compared with that seen in uninfected mice (FIG. 15). Exposure of (HDK-1 DNAx Research Institute) T cells (a Th1 type of T cell) in vitro to PADES inhibits the release of IL-2, IL-3 and γ-IFN (cytokines released by Th1 cells; Table 1).
Table 1.
Inhibition of secretion of γ-IFN, IL-2 and IL-3 by HDK-1 cells exposed in vitro to Trichinella pseudospiralis products exuded by pre-adult worms.

<table>
<thead>
<tr>
<th>CYTOKINE</th>
<th>% OF CONTROLS</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-IFN</td>
<td>20.27%</td>
<td>15</td>
</tr>
<tr>
<td>IL-3</td>
<td>26.44%</td>
<td>15</td>
</tr>
<tr>
<td>IL-2</td>
<td>25.45%</td>
<td>7</td>
</tr>
</tbody>
</table>

T. pseudospiralis is modulating the host’s immune response at this very basic level, and by using a molecule that mimics IL-10, is directing host reaction in a manner that best promotes its own survival as well as that of the host. There are some basic structural and presumably functional differences between the parasites TP10 and host IL-10. Commercially prepared monoclonal antibody to mammalian IL-10 (DNAX Research Institute) does not cross react with TP10. However, serum from hosts infected with T. pseudospiralis does cross react with recombinant mammalian IL-10 and interfere with the ELISA for IL-10 (See Table 2 and FIG. 16).
Table 2.
The effects of 50 µl of sera from Trichinella pseudospiralis-infected mice on detection of rIL-10 using an ELISA for IL-10.

<table>
<thead>
<tr>
<th>rIL-10 Units/ml</th>
<th>O.D. w/o Sera</th>
<th>O.D. w/ Sera</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>1.208</td>
<td>0.621</td>
<td>4</td>
</tr>
<tr>
<td>100</td>
<td>0.951</td>
<td>0.408</td>
<td>4</td>
</tr>
<tr>
<td>33</td>
<td>0.558</td>
<td>0.179</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>0.253</td>
<td>0.062</td>
<td>4</td>
</tr>
<tr>
<td>3.3</td>
<td>0.094</td>
<td>0.016</td>
<td>4</td>
</tr>
<tr>
<td>1.1</td>
<td>0.040</td>
<td>0.004</td>
<td>4</td>
</tr>
</tbody>
</table>

The IL-10-like factor elaborated by preadult

T. pseudospiralis inhibits secretion of IL-2, IL-3 and γ-IFN by T cells as shown in Table 3. HDK-1 (CD4+ Th1 cell) cells were cultured in vitro at 37°C under 5% CO₂ in RPMI 1640 containing 10% fetal calf serum, antibiotics, 2-mercaptoethanol and L-glutamine (GIBCO, Grand Island, NY; JRH Biosciences, Lenexa, KS). Cytokine assays were performed on supernatants derived from cultures of HDK-1 cells following 24 hr exposure to antigen-presenting cells (x-irradiated Balb/c spleen cells [3000 rads]) and antigen (KLH) and either (1) rIL-10 at 40 units/well (positive controls), (2) 50 µl of medium alone. IFN-γ and IL-3 were measured by two-site sandwich ELISA (Mosmann, et al. (1989, and (1987)). IL-2 was assayed using the method of Cherwinski et al. (1987). Preadult and adult ES were generated as described in Example 2. Data are presented as mean percent inhibition of cytokine secretion. Standard deviation and n are presented for each mean. Preadult ES and rIL-10 differed significantly from controls (P<0.01).
Table 3.

Preadult ES Inhibit Th1 Cytokine Release

<table>
<thead>
<tr>
<th>Factors</th>
<th>γ-IFN</th>
<th>IL-2</th>
<th>IL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>rIL-10</td>
<td>76 ± 7  (17)</td>
<td>ND</td>
<td>55 ± 11 (17)</td>
</tr>
<tr>
<td>Preadult ES</td>
<td>85 ± 22 (23)</td>
<td>49 ± 27 (5)</td>
<td>82 ± 11 (15)</td>
</tr>
</tbody>
</table>

Presented in Table 3 are data from a typical study in which the effects of preadult ES on cytokine release by a Th1 cell line were assessed. TP10 in PAD ES showed greater potency for suppressing Th1 cytokine release than did mammalian rIL-10 (200 IU/ml), significantly lowering the release of gamma interferon, IL-2 and IL-3. Cytokines were assessed as indicated above.

The first indication of systemic modulations in cytokine patterns occurs during the newborn larval phase of infection (days 6-12 postinfection), as found when mesenteric lymph node (MLN) cells and spleen cells isolated from T. pseudospiralis-infected mice were examined. IL-2 secretion by MLN cells and spleen cells recovered from infected mice was increased by almost 225% and 450% respectively by day 10 postinfection, as shown in FIG. 17. Blood for cytokine analysis was obtained by cardiac puncture from uninfected mice and from mice infected with T. pseudospiralis for 10 days was treated according to conventional methods for recovery of serum. Peritoneal exudate for analysis of cytokines was obtained from mice infected for 10 days with T. pseudospiralis and from uninfected mice by peritoneal lavage using sterile RPMI 1640 (GIBCO). Likewise, IL-2 levels in serum and the peritoneal cavity in mice infected for 10 days were found to be 1,200% and 1,000% of controls respectively. Although newborn larval ES itself showed no IL-2 activity, this material elicited secretion by HDK-1 cells
in vitro of a substance with IL-2-like activity capable of stimulating proliferation of an IL-2-dependent cell line (HT2 cells) by over 8,000% above controls (Table 4). By contrast exposure of HDK-1 cells to preadult or adult ES did not induce secretion of this IL-2-like activity.

Stewart et al. (1988) showed a dramatic rise in the levels of plasma corticosterone in the T. pseudospiralis-infected host and that this elevation accompanied a systemic and non-specific hypothesized that the parasite may release material which stimulates release by host cells of key cytokines that serve as molecular messages between the immune and neuroendocrine systems of the host. This parasite-induced hypersecretion of host cytokines would cause activation of the HPA axis with subsequent release by the adrenals of increased amounts of corticosterone. The fact that the elevation in plasma corticosterone extends well into the muscle phase of infection indicates that the muscle larvae must participate in this event.

In situ Hybridization Analysis. Since newborn larval ES appear to ultimately stimulate release of copious amounts of IL-2 in vivo (FIG. 17), samples of diaphragm muscle from T. pseudospiralis-infected and from uninfected mice were probed for IL-2 mRNA by in situ hybridization using an \(^{35}\)S-labeled probe for murine IL-2 (FIG. 18). Normal mouse diaphragm muscle shows a diffuse background reaction (FIG. 18A), while infected muscle shows very intense, localized reaction in connective tissue elements (FIG. 18B). The inset in this FIG. demonstrates that larvae (L) do not express IL-2 mRNA above background.

Cells of the immune system are often found in the connective tissue of muscle in a diseased state, but the relative paucity of immune response in T. pseudospiralis
infections of muscle and the intensity of reaction suggested that fibroblasts might also be a source of the mRNA detected in the connective tissue of infected muscle. Thus, 3T3 murine fibroblasts (ATCC) were exposed in vitro to newborn larval ES and the supernatant was examined for IL-2 using an HT2 (IL-2 dependent) cell line. 3T3 murine fibroblasts were maintained in complete DMEM (see above) at 37°C under 5% CO₂. For analysis of IL-2 secretion by 3T3 fibroblasts exposed to newborn larval ES, monolayers were established in 24-well plates to which parasite ES was added (100μg/ml). Forty-eight hr following addition of parasite ES, culture supernatants were collected and assessed for IL-2 activity using the HT2 cell proliferation assay of Cherwinski et al. (1987). Based on this assay, 3T3 culture supernatants induced HT2 proliferation as high as 20,000% above controls (Table 5). However, as with the HDK-1 cells stimulated with newborn larval ES (Table 4), IL-2 was not detectable by ELISA of culture supernatants. Thus, it was concluded that newborn larval ES were inducing release by both fibroblasts and T cells, in vivo and in vitro, of an IL-2-like substance that stimulated proliferation of T cells of the Th1 subset. This activity in the intact animal leads to release of IL-2, as might any potent activator of Th1 cells. In one study, exposure of 3T3 fibroblast in vitro to live newborn larvae for 24 hr caused release by 3T3 cells of IL-2-like activity in excess of 500,000% of controls.
Table 4.
Secretion of IL-2-like activity by HDK-1 cells exposed to Trichinella pseudospiralis newborn larval ES (NBL ES), adult ES (AD ES), preadult ES (PAD ES) and live newborn larvae (NBL).

<table>
<thead>
<tr>
<th>TEST MATERIAL</th>
<th>% OF CONTROLS</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBL ES</td>
<td>8.175%</td>
<td>16</td>
</tr>
<tr>
<td>Ad ES</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>PAD ES</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>NBL</td>
<td>4.253%</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 5.
Release of IL-2-like activity in vitro by 3T3 mouse fibroblasts exposed to Trichinella pseudospiralis newborn larval ES.

<table>
<thead>
<tr>
<th>Well #</th>
<th>% of Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.086%</td>
</tr>
<tr>
<td>2</td>
<td>20.034%</td>
</tr>
<tr>
<td>3</td>
<td>12.600%</td>
</tr>
<tr>
<td>4</td>
<td>3.281%</td>
</tr>
<tr>
<td>5</td>
<td>4.942%</td>
</tr>
</tbody>
</table>

Previous studies have shown that during the migratory phase of the newborn larvae and early muscle larval phase of infection with T. pseudospiralis, hosts display greatly enhanced natural killer (NK) cell activity (Niederkorn, et al., 1988). TPI directed the stimulation of in vivo NK cell activity, as shown in Table 6, of mice injected with supernatants from 3T3 murine fibroblasts stimulated with newborn larval ES. Pulmonary NK cell activity was assessed by an in vivo
clearance assay with NK-sensitive tumor target cells (YAC-1 lymphoma). $^{51}$Cr-labeled tumor cells ($10^6$) were intravenously injected into groups of treated and untreated mice. Clearance of $^{51}$Cr-labeled tumor cells was determined by counting the radioactivity in lungs removed from 8-10 wk-old, female C57/BL6 mice (Jackson Laboratories, Bar Harbor, ME) 2.5 hr after tumor cell injection. NK cell activity is inversely related to the radioactivity in the lungs; that is, the higher the radioactivity the lower the NK cell-mediated clearance. Clearance is expressed as % enhanced clearance and is calculated as follows: % enhanced clearance = [1 - (mean cpm in test lungs/mean cpm in control lungs)] x 100. The data were normalized for each pair of test and control groups so that controls were designated as producing 0% enhanced clearance (i.e. background level). Mice were untreated (Naive), injected intraperitoneally daily for 2 days with either 0.5 ml of supernatant from 3T3 cultures not exposed to newborn larval ES (Medium Only) or 0.5 ml of supernatant from 3T3 cultures exposed for 48 hr to newborn larval ES (100 μg parasite protein/ml of culture medium). Mice injected intraperitoneally at 18-24 hr before assay with 100 μg/mouse of Poly I:C (a known stimulator of NK activity) in RPMI 1640 served as positive controls. Each group contained 4 or 5 animals.

Table 6.

<table>
<thead>
<tr>
<th>In Vivo Stimulation</th>
<th>CPM in Lungs±SEM</th>
<th>% Enhancement</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>15,572±3,878</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium</td>
<td>14,470±2,486</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Poly I:C</td>
<td>12,924±3,510</td>
<td>17</td>
<td>NS</td>
</tr>
<tr>
<td>Newborn Larval ES</td>
<td>10,895±1,952</td>
<td>30</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
IL-2 Secretion Assay. To measure IL-2 secretion by lymphoid cells during the newborn larval phase of infection with *T. pseudospiralis*, spleen and MLN cells were collected from noninfected (control) and infected female C57/BL6 mice (Jackson Labs, Bar Harbor, ME) on days 6, 10, 17 or 24 following infection with *T. pseudospiralis*. 5x10^6 spleen or MLN cells were added to individual wells in microtiter plates and stimulated with Con A (5 μg/well). Culture supernatants were collected 24 hr later and the IL-2 activity quantified in a conventional bioassay using HT2 cells following the method of Cherwinski et al. (1987) and by application of an IL-2 specific ELISA. IL-2 secretion is represented as the mean percent of normal control. Each bar represents the mean of 16 separate samples. IL-2 detected in culture supernatant of spleen cells and MLN cells recovered from infected mice on day 10 was significantly greater than that for controls and for days 7, 17 and 24 postinfection (P<0.01) as shown in FIG. 17.

A number of hosts are highly resistant to infection with *T. spiralis*. In one of these hosts, the Chinese hamster, the basis for resistance to this parasite is an extremely rapid and intense inflammatory response mounted during the early stages of infection. When Chinese hamsters were coinfected with both parasites, *T. pseudospiralis* suppressed the inflammatory response normally mounted by this host. This provided levels of success for *T. spiralis* infection normally seen only in the most permissive of hosts (FIG. 5). Inflammatory response at host sites normally occurring when *T. spiralis* was present alone, was dramatically reduced in the presence of *T. pseudospiralis* (as seen in FIG. 6; and by Larsen, Stewart and Niederkorn 1991).
EXAMPLE 2
PURIFICATION OF TP10 AND TPI

The purification of a Trichinella pseudospiralis IL-10-like factor (TP10) and an IL-2-like activity inducing factor (TPi), as described in the preferred embodiments and as presented in Example 1, may be isolated in the following manner.

METHODS
1. Purification of TP10.
   Mice infected with the muscle stage of this parasite were skinned and eviscerated, homogenized in a Waring blender in 250 ml/mouse of 1% pepsin-1% HCl solution at high speed for two 15 sec bursts. The resulting homogenate was incubated in beakers at 37°C for 1 hr in a water bath shaker. Incubates were allowed to sediment for 20 min at room temperature and the supernatant removed by aspiration. The larvae were washed twice with PBS by sedimentation and forced through 16 layers of cheesecloth using a stream of PBS. The larvae were placed in glass incubation chambers containing RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2% antibiotic/antimycotic, L-glutamine and 10 mM HEPES buffer (approximately 100,000 larvae/50 ml of medium) and incubated for 48-72 hr at 37°C in a serological water bath. Fetal calf serum may be left out for incubations of 24-48 hr. Larvae were removed by centrifugation at 1000 rpm for 5 min and the supernatant containing the ES was retained.

   The ES supernatant was passed through a sterile 0.22 μm filter, and concentrated five times using a 10kD membrane in an ultrafiltration apparatus. The resulting material was aliquoted in microfuge tubes and stored at -70°C until needed. Purified TP10 is defined as freed from whole or viable T. pseudospiralis.
The purification of a Trichinella pseudospiralis inducer of IL-2-like activity inducing factor, as described in the preferred embodiments and with the characteristics described in Example 1 may be isolated in the following manner.

2. Purification of TPi

For purification of TPi, adult worms were obtained from rats that had been infected with 20,000 infective T. pseudospiralis larvae, per host. The T. pseudospiralis larvae were isolated from mouse muscle by methods described by Stewart et al. (1990). The entire small intestine of each rat was removed on day 6 postinfection washed out with saline-2% antibiotic/antimycotic (streptomycin [10,000 µg/ml]-penicillin [10,000 units/ml]-fungizone [amphotericin B; 25 µg/ml]; AA) using a syringe, split lengthwise and placed in saline-1% AA (100ml/5 guts) at 37°C for 1 hr in a serological water bath. Adult worms were allowed to migrate out of the intestinal wall into the saline. Intestines and debris were removed by passing the worm suspension through a series of sieves and the worms were washed three times with saline-1% AA by centrifugation. Alternatively, adult worm suspensions were passed through a Whatman #4 filter pad mounted in a filter holder with a mild suction applied. Worms and debris adherent to the filter pad were separated by floating the filter pad worm-side down in a shallow bowel containing PBS-1% AA to a depth of 2 cm and placed in a 37°C serological water bath for 30 min to allow the worms to migrate off the filter pad, leaving debris behind. The isolated worms were washed twice in RPMI 1640 supplemented as indicated above (except that 30% FCS was used and approximately 25,000 worms were incubated in each 50 ml of medium). Adult worms were incubated in the above medium in covered glass dishes for 24-72 hr for release of newborn larvae (NBL). Following deposition of NBL, worm suspensions were pooled and
passed through a 325 mesh brass sieve (Baxter, Grand Prairie, TX) to remove adult worms. NBL were washed 5 times in RPMI 1640 supplemented as indicated above (except that 30% FCS was used and approximately 25,000 worms were incubated for an additional 48 hr. Following incubation, NBL were removed by centrifugation through a sterile 0.22 μm filter and concentrated as above for PAD ES and stored at -75°C until needed. Only ES of NBL were present in these supernatants. Purified TPI is defined as freed from whole or viable T. pseudospiralis.

The closely related parasite, Trichinella spiralis, also appears to elaborate at least one substances with effects similar to those shown for TP10 and TPI. Many of the Inventor's studies on T. pseudospiralis (TP) have been comparative in nature, with T. spiralis (TS) run in parallel as a control infection. In practically all cases, TS appears to employ immunoevasive strategies similar to those used by TP. However, TP consistently has a much greater influence over targeted immunological functions in the host. Examples from past studies by the Inventor would include: 1) suppression of inflammation to unrelated antigen (e.g., cotton string implants; Stewart et al., 1985); 2) host pleural natural killer cell activity (Niederkorn et al., 1988); 3) elevation in plasma corticosterone; 4) induction of secretion by NBL ES of material having IL-2-like activity by 3T3 murine fibroblasts and HDK-1 cells in vitro (unpublished); 5) expression of host asialo GM1 antigen by muscle larvae (Niederkorn et al., 1988; and, 6) suppression of Th1 cytokine release by preadult ES (unpublished). In all cases except #5, TP has a significantly greater impact on the immunological function in question. These differences between the two parasites fit well with differences in their biology. TS is encapsulated in the muscle and need not exert control over myositis for its own protection. It must however, not allow myositis to
kill the host and therefore may raise corticosterone levels to a sufficient degree to avoid extreme inflammatory reaction against the muscle phase. If this immunopathological mechanism were not controlled it might compromise the muscles involved in respiration or precipitate a fatal myocarditis. Indeed, these are among the primary causes of death in trichinosis, but not in infections with TP. The greater importance of control over the neuroendocrine system of the host for survival of both the host and TP comes from adrenalectomy studies. Here, 100% of adrenalectomized, TP-infected mice died by day 16 postinfection. All such mice could be salvaged by injection of cortisone acetate on a daily basis. However, no adrenalectomized mice infected with TS died during these studies (Stewart et al., 1988).

The adult worm of TS residing in the gut of the host bowel generally displays much greater reproductive potential in most hosts than does TP. Yet TS is much more pathogenic than is TP. However, TS induces significantly higher levels of enteritis in its hosts than does TP. If TS were to exert the same degree of control over the development of enteritis displayed by TP, it would extend the duration of its adult phase and increase its fecundity (enteritis has a direct negative effect on the longevity and fecundity of adult Trichinella; (Stewart, G.L., et al. (1982)) which would cause an increase in morbidity and mortality associated with the stage of muscle invasion. Indeed, administration of cortisone to TS-infected mice at levels equivalent to those reached naturally during the course of infection with TP causes a dramatic increase in morbidity and mortality from both the intestinal and muscle phases of infection with TS.

Nevertheless, both encapsulated and unencapsulated forms of Trichinella appear capable of modulating
immunity by mechanisms similar to those described in this document.

* * *

5 All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.
REFERENCES:

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.


CLAIMS

1. A purified Trichinella-derived substance having certain immunomodulatory characteristics of IL-10, said characteristics including a Th1 immunosuppressive effect.

2. The substance of claim 1 defined further as derived from Trichinella pseudospiralis.

3. The substance of claim 1, further defined as derived from the pre-adult larvae of Trichinella pseudospiralis.

4. A purified IL-10-like substance having the following characteristics:

   isolatable from pre-adult Trichinella exudates;

   said exudates having a molecular weight of between 100-300 kD; and

   said exudates being immunosuppressive to Th1 phenotype T cells.

5. A method of obtaining a purified IL-10-like substance from Trichinella pseudospiralis comprising the steps of:

   obtaining Trichinella pseudospiralis-organisms;

   incubating said organisms under conditions facilitating the exudation of an IL-10-like substance; and
purifying a *Trichinella* derived IL-10-like substance from said incubate by separating intact organisms or fragments thereof.

6. The method of claim 5, wherein the purifying of step (c) is accomplished separating intact organisms or fragments thereof through a 0.2μm filter.

7. The method of claim 5, wherein the purification of step (c) is accomplished by ultrafiltration with a molecular weight cut-off of between about 10 kDa to 100 kDa.

8. The method of claim 5, wherein the purification of step (c) is accomplished by ultrafiltration with a molecular weight cut-off of about 10 kDa.

9. An antibody having a specific binding affinity for an IL-10-like substance produced by a member of the *Trichinella* sp..

10. A method of purifying an IL-10-like substance having the ability to stimulate T cells toward a Th2 phenotype and away from a Th1 phenotype, comprising obtaining an exudate from freshly isolated or cultured nematodes, and retrieving an IL-10-like substance from said exudate with an antibody having a specific binding affinity for the IL-10-like substance.
11. A purified Trichinella-derived IL-2-inducing substance characterized as, eliciting release of an IL-2-like activity by eukaryotic cells.

12. The substance of claim 11, defined further as being derived from *Trichinella pseudospiralis*.

13. The substance of claim 11, defined further as being derived from the newborn stage of *Trichinella pseudospiralis*.

14. A purified IL-2-inducing substance having the following characteristics:

    isolatable from newborn *Trichinella* exudates;

    having a molecular weight of 30-50 kD; and

    inducing secretion of IL-2 by eukaryotic cells.

15. A method of obtaining a purified IL-2-inducing substance comprising the steps of:

    obtaining *Trichinella pseudospiralis*-organisms;

    incubating said organisms under conditions facilitating the exudation of an IL-2-inducing substance; and

    purifying a *Trichinella* derived IL-2-inducing substance from said incubate by separating intact organisms or fragments thereof.
16. The method of claim 15, wherein the purifying of step (c) is accomplished separating intact organisms or fragments thereof through a 0.2μm filter.

17. The method of claim 15, wherein the purification of step (c) is accomplished by ultrafiltration with a molecular weight cut-off of between about 10 kDa to 30 kDa.

18. The method of claim 15, wherein the purification of step (c) is accomplished by ultrafiltration with a molecular weight cut-off of about 10 kDa.

19. An antibody having a specific binding affinity for a IL-2-inducing substance produced by a member of the Trichinella sp..

20. A method of purifying an IL-2-inducing substance able to stimulate eukaryotic cells to secrete an IL-2-like activity, comprising: obtaining an exudate from freshly isolated or cultured Trichinella and retrieving an IL-2-inducing substance from said exudate with an antibody having a specific binding affinity for said IL-2 inducing substance.

21. A method of early prognosis of Trichinella pseudospiralis infection comprising:

    identifying a human subject suspected of being infected with Trichinella; and
assaying a blood sample of said subject for the presence of TP10 or TPi.

22. A pharmaceutical composition comprising the substance of claim 1 or 11, dispersed in a pharmacologically acceptable carrier.

23. An improved and novel method for suppressing an immune response comprising treating eukaryotic cells with an effective amount of a pharmaceutical composition; wherein said cells are contacted with a composition in accordance with claim 22.

24. A method for altering the immune response of an animal comprising the following steps:

preparing a pharmaceutical composition comprising the substance of claim 1 or claim 11, dispersed in a pharmacologically acceptable carrier; and

administering to an animal a therapeutically effective amount of said substance.

25. A method for treating an animal comprising:

identifying an animal with a need for immunosuppression;

administering to an animal a therapeutically effective amount of the substance of claim 1 or claim 11, dispersed in a pharmacologically acceptable carrier;
wherein the immune response being suppressed is a graft versus host response, an autoimmune response or an allergic response.
FIG. 1

FIG. 2
FIG. 10
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

<table>
<thead>
<tr>
<th>IPC(6)</th>
<th>US CL</th>
<th>According to International Patent Classification (IPC) or to both national classification and IPC</th>
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<td>C07K 14/44</td>
<td>435/71.1, 71.2; 530/351, 412, 820, 822.</td>
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**B. FIELDS SEARCHED**

<table>
<thead>
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<th>Minimum documentation searched (classification system followed by classification symbols)</th>
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<tbody>
<tr>
<td>U.S.</td>
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</tbody>
</table>

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

- APS, CAS ONLINE, BIOSIS, EMBASE, MEDLINE, WPIDS, JICST-E.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>US, A, 4,404,194 (ARALA-CHAVES) 13 September 1983, see whole document, particularly col. 1, lines 43-52, col. 6, line 59.</td>
<td>1-8</td>
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<tr>
<td>X</td>
<td>Biological Abstracts, Volume 91, Number 7, issued 01 April 1991, Hong et al., &quot;Sero logical cross reactions between saline extract of Trichinella spiralis muscle larvae and human sera infected with trematodes&quot;, page 874, column 1, abstract no. 76503, Chung-ang Journal of Medicine, Volume 15, No. 2, issued 1990, pages 197-208, see entire abstract.</td>
<td>1-4</td>
</tr>
</tbody>
</table>

- Further documents are listed in the continuation of Box C.
- See patent family annex.

<table>
<thead>
<tr>
<th>Special categories of cited documents:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - document defining the general state of the art which is not considered to be of particular relevance</td>
</tr>
<tr>
<td>E - earlier document published on or after the international filing date</td>
</tr>
<tr>
<td>L - document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td>
</tr>
<tr>
<td>O - document referring to an oral disclosure, use, exhibition or other means</td>
</tr>
<tr>
<td>P - document published prior to the international filing date but later than the priority date claimed</td>
</tr>
</tbody>
</table>

**Date of the actual completion of the international search**

- 20 JUNE 1995

**Date of mailing of the international search report**

- 2 9 JUN 1995

**Name and mailing address of the ISA/US**

- Commissioner of Patents and Trademarks
- Box PCT
- Washington, D.C. 20231

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- (703) 305-3230

**Authorized officer**

- C. SAYALA

**Telephone No.**

- (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)
INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-8

Remark on Protest [ ] The additional search fees were accompanied by the applicant’s protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)
BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-8, drawn to an immunomodulatory factor (IL10 like) and its purification.
II. Claims 9-10, drawn to an antibody to an IL10 like factor and its use in purification.
III. Claims 11-18, drawn to an inducer of IL2 secretion and its purification.
IV. Claims 19-20, drawn to an antibody to an inducer of IL2 secretion and its use in purification.
V. Claim 21, drawn to assays for an inducer of IL2 secretion (TPI), in the second recited embodiment of claim 21.
VI. Claims 22-25, drawn to pharmaceutical compositions containing an IL10 like factor and their use in body treatments, in the first recited embodiment of claims 22-25.
VII. Claims 22-25, drawn to pharmaceutical compositions containing an inducer of IL2 secretion and their use in body treatments, in the second recited embodiment of claims 22-25.

The compositions of Groups I and III are drawn to different immunomodulatory factors that have different core structures, physical/chemical properties and physiological functions. There is thus no common technical feature to link these. Since the compositions of Groups I and III are different, the antibodies to each of these (Groups II and IV) must also be different from each other (i.e. have different variable regions and different combining specificities). These antibodies thus have no common technical feature to link these to each other.

The compositions of Groups I and III and their antibodies (Groups II and IV) are each distinct compositions with different core structures (e.g. the immunomodulatory factor and the antibody thereto have no common sequence). The antibodies are not used in any purification step recited in Groups I and III. There is thus no common technical feature that links the compositions (Groups I and II) and the antibodies thereto (Groups II and IV).

The method of assay for TPI in Group V is the second recited use of the compositions in the claims (note the first recited assay method for TP10 in claim 21 would be placed with the composition of Group I, since unity of invention permits one composition, the first recited method of its preparation, and the first recited method of its use).

The compositions and treatment of Group VI pertain to the second recited use of the first recited composition in the claims.

The compositions and treatment of Group VII pertain to the second recited use of the second recited composition in the claims.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.