Modification Form for Permit BIO-UWO-0066

Permit Holder: Bhagirath Singh

Approved Personnel
(Please stroke out any personnel to be removed)

~~Katrina Huszarik~~

~~Jordan Schwartz~~

Enayat Nikoopour

Olga Krougly

Edwin Lee Chan

~~Hui Yu Qin~~

Additional Personnel
(Please list additional personnel here)

Christian Sandrock.

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	BCG Mycobacterium bovis, E. coli (DH5 alpha)	
Approved Cells	Human blood, rodent T-cells, rodent dendritic cells, U937, PU5, NIT	
Approved Use of Human Source Material	blood (whole, synovial fluid), blood (fraction serum cells), tissues (unpreserved synovial tissue)	
Approved GMO	SV 40 Large T antigen NIT cells	
Approved use of Animals	mice, rabbits, rats	
Approved Toxin(s)	Staphylococcal Enterotoxin B	Pertussis Toxin

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: _____

Classification: 2

Date of Last Biohazardous Agents Registry Form: Aug 24, 2007

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Questions.

1Q. How much do you plan to use at once?

2Q. How much will you keep on hand (ie stored)?
Where will it be stored (ie locked fridge, etc.)?

3Q. What is the LD50 (and species, estimated for human if possible) for the toxin?

We will also need a description of the work...

Answers to Questions:

1A. 4μg per experiment

2A. We will have NO more than 60μg of pertussis toxin at any given time in the Lab.

Storage: Reconstitute one vial of 50μg in 0.5ml saline (100X Stock solution). Store 40μl aliquotes at –20°C in a un-lock freezer in Dr. Bhagirath Singh's Laboratory in SDRI room 224, which is locked after hours/if no one is present.

3A. LD50 intravenous in rats: 0.114 mg/kg from MSDS

Description of experimental autoimmune enchepelemyletis (EAE) work

Pertussis toxin (PT) diluted to 1ng/μl. 200μl (200ng) will be injected intraperitoneally into mice to weaken the blood brain barrier and facilitate rapid induction of experimental autoimmune enchepelemyletis (EAE).

NOD Mice for these series of experiments will be divided into 2 groups of 10 mice. Induced donors and Recipient mice.

Induced donors will be injected with MOG peptide subcutaneously at four locations on their back. At this time they will also be injected intraperitonealy (IP) with pertussis toxin(PT) 200ng in 200ul/mouse, a second injection of PT will be administered 2 days after the first. The mice will be monitored daily for behavioural changes starting on day 7 post injection. After 9-14 days mice will be euthanized and tissues harvested for experiments.

Recipient mice will receive a transfer of treated immune cells intravenously via tail vein. Recipient mice will also receive pertussis toxin (PT) injections (200ng in 200ul/mouse) intraperitonealy (IP) on the day 0 and 2 post transfer. Mice will be monitored starting on day 4 post transfer for weight loss and behavioural changes, which will be scored acquiring to guidelines for 60 days.

All waste, bedding and carcases will be incinerated. Cages will be chemically treated and autoclave.

Canadian Centre for Occupational Health and Safety

CCOHS

RTECS Registry of Toxic Effects of Chemical Substances®

Data source: MDL Information Systems, Inc.

CHEMICAL IDENTIFICATION

RTECS Number	XW5883750
Chemical Name	Toxins, pertussis
CAS Registry Number	70323-44-3
Other CAS Registry Nos.	82248-93-9
Last Updated	200808
Data Items Cited	9
Compound Descriptor	Drug Natural Product

Synonyms/Trade Names

IAP
Lymphocytosis-promoting factor
Pertussigen
Histamine-sensitizing factor
Islet activating protein

HEALTH HAZARD DATA

ACUTE TOXICITY DATA

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent - rat	114 ug/kg	Sense Organs and Special Senses (Eye) - lacrimation Behavioral - changes in motor activity (specific	TJEMAO Tohoku Journal of Experimental Medicine. (Maruzen Co. Ltd., Export Dept., P.O. Box 5050, Tokyo Int., 100-31 Tokyo, Japan) V.1- 1920- Volume

				assay) Nutritional and Gross Metabolic - weight loss or decreased weight gain	(issue)/page/year: 130,105,1980
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent - mouse	17160 ng/kg	Details of toxic effects not reported other than lethal dose value	INFIBR Infection and Immunity. (American Soc. for Microbiology, 1913 I St., NW, Washington, DC 20006) V.1- 1970- Volume (issue)/page/year: 31,495,1981
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent - mouse	127 ug/kg	Sense Organs and Special Senses (Eye) - lacrimation Behavioral - changes in motor activity (specific assay) Nutritional and Gross Metabolic - weight loss or decreased weight gain	TJEMAO Tohoku Journal of Experimental Medicine. (Maruzen Co. Ltd., Export Dept., P.O. Box 5050, Tokyo Int., 100-31 Tokyo, Japan) V.1- 1920- Volume (issue)/page/year: 130,105,1980
TDLo - Lowest published toxic dose	Intracerebral	Rodent - mouse	200 ng/kg	Biochemical - Metabolism (Intermediary) - other	JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams & Wilkins Co., 428 E. Preston St., Baltimore, MD 21202) V.1- 1909/10- Volume (issue)/page/year: 299,960,2001
TDLo - Lowest published toxic dose	Intracerebral	Rodent - mouse	10 ug/kg	Behavioral - changes in psychophysiological tests	NEROEW Neuropsychopharmacology. (Elsevier Science, 655 Avenue of the Americas, New York, NY 10010) V.1- 1987- Volume (issue)/page/year: 27,554,2002
TDLo - Lowest published toxic dose	Intravenous	Rodent - rat	30 ug/kg	Vascular - other changes	EJPHAZ European Journal of Pharmacology. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1967- Volume(issue)/page/year: 493,139,2004
TDLo - Lowest published toxic dose	Intravenous	Rodent - rat	15 ug/kg	Vascular - BP lowering not characterized in autonomic section	EJPHAZ European Journal of Pharmacology. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1967- Volume(issue)/page/year: 493,139,2004
TDLo - Lowest published toxic dose	Subcutaneous	Rodent - mouse	333.333 ng/kg	Biochemical - Metabolism (Intermediary) - effect on inflammation or	JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams & Wilkins Co., 428 E. Preston St.,

				mediation of inflammation	Baltimore, MD 21202) V.1- 1909/10- Volume (issue)/page/year: 318,611,2006
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - rat	15 ug/kg	Vascular - contraction (isolated tissues)	NSAPCC Naunyn-Schmiedeberg's Archives of Pharmacology. (Springer Verlag, Heidelberger, Pl. 3, D-1000 Berlin 33, Fed. Rep. Ger.) V.272- 1972- Volume(issue)/page/year: 369(Suppl 1),R171,2004

END OF RECORD

RTECS® is provided quarterly by MDL Information Systems, Inc. and was last updated: **2009-05**.

Experimental Autoimmune Encephalomyelitis (EAE)

In the United States, it is estimated that 250,000 to 350,000 people have physician-diagnosed multiple sclerosis (MS). The majority of patients are diagnosed with the disease during their third and fourth decade of life, resulting in many patients suffering the effects of this disease for most of their adult life. The cause of MS is unknown. However, because the disease is characterized by perivascular, inflammatory cell infiltrates and demyelination, features also characteristic of experimental autoimmune encephalomyelitis (EAE), an autoimmune process is thought to be involved in disease pathogenesis. Recently, three drugs, interferon-β1a, interferon-β1b, and glatiramer acetate have been approved by the FDA for use in the treatment of patients with relapsing and remitting MS. However, while these agents reduce exacerbations and slow the clinical progression of MS, they are not a cure for the disease, therefore, the need for better treatment strategies in MS remains.

There are several animal models that have been used to study MS. In some of these models, disease is induced by viruses such as Theiler's virus or Borna virus. Of the EAE models, the most commonly studied are those established in the Lewis rat and in several susceptible mouse strains. The procedures described in this unit utilize the murine models exclusively for a number of reasons. Murine EAE often results in a relapsing/remitting disease, similar to the early phase of most MS patients, while EAE in the Lewis rat is a monophasic illness in which animals experience a single episode of paralysis from which most recover completely. In chronic murine EAE, the pathology observed in the white matter shows much more demyelination than the Lewis rat model, again being more reminiscent of the pathology seen in the CNS of patients with MS. With the advent of transgenic and homologous recombination technology, it is becoming increasingly clear that many powerful molecular tools are becoming available to study the immune response and subsequent CNS pathology in mice in inflammatory processes such as EAE. In addition, the fact that axonal pathology is noted in both EAE and MS will likely result in the model being used by neuroscientists as new neuroprotective strategies are developed for these disorders.

NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) or must conform to governmental regulations regarding the care and use of laboratory animals.

STRATEGIC PLANNING

When choosing a model of EAE, the most important issue is defining the question being addressed by the investigator. An investigator may wish to know whether a newly developed transgenic or "knock out" mouse is susceptible to disease. In such a case, the goal would be to initiate the disease in the simplest manner possible. Others may be more interested in testing a new pharmacologic compound for therapeutic efficacy in the model. In this case, the investigator may not only be interested in whether the compound can prevent the development of EAE, but also may wish to know whether the compound is efficacious in treating established disease in chronic, relapsing models. This section discusses the major subtypes of the EAE model and the strengths of the different models.

EAE is divided into two major subtypes, (1) active EAE (see Basic Protocol) and (2) passive or adoptively transferred EAE (see Alternate Protocol). In active EAE, susceptible strains of mice are immunized with an appropriate myelin antigen or peptide emulsified in complete Freund's adjuvant (CFA). Mice also are usually administered pertussis toxin

Contributed by Michael K. Racke
Current Protocols in Neuroscience (2001) 9.7.1-9.7.11

(PT) on the day of immunization and 48 hr later. PT is thought to play a role in the breakdown of the blood-brain-barrier and recent studies also suggest that PT increases interferon-γ production by encephalitogenic T cells. Depending on the strain of mouse and the antigen used, EAE will manifest 10 to 15 days after the initial immunization. With active immunization, 75% to 80% incidence of disease can be expected. Very often with active disease, the course of disease is monophasic. An important exception is the active immunization model induced in SJL or (SJL × SWR) F1 mice using the proteolipid protein (PLP) peptide 139-151 emulsified in CFA as the inducing antigen. In this instance, the mice develop a relapsing and remitting disease course and this occurs without the use of PT.

In passive or adoptively transferred EAE, the disease is induced by injecting mice with activated, myelin-specific T cells, which then causes these animals to develop EAE. These activated T cells are generated by immunizing mice with the inducing myelin protein or peptide emulsified in CFA. The draining lymph nodes are removed and the T cells activated again with the inducing antigen in vitro. The activated T cells are then transferred into naive recipients, which then develop EAE. The most common forms of adoptively transferred EAE are in the SJL or (PL × SJL) F1 mouse using myelin basic protein (MBP) as the inducing antigen (e.g., Racke et al., 1992). In these two forms of EAE, the mice develop a relapsing and remitting form of disease, which makes this model very conducive to studies examining interventions for established disease. In addition, another advantage to the adoptive transfer model is that EAE can be broken down into its various pathophysiologic components. These components include the initial priming of encephalitogenic T cells in donor mice that have been immunized with the myelin antigen, the subsequent activation and expansion of encephalitogenic T cells in vitro, and the subsequent capability of these T cells to enter the CNS and cause the clinical manifestations of EAE. If an experimental therapeutic agent prevented the priming of encephalitogenic T cells, it probably would not be effective if given to recipient mice that have received T cells that were already primed and activated.

With the advent of transgenic technology, mice have been generated that have the majority of their T cells specific for myelin antigens. The mice that have been most thoroughly characterized in this regard are those with T cells specific for the N-terminal epitope of MBP, Ac1-11 (Goverman et al., 1993). This strain is available from the Jackson Laboratory and allows study of the adoptive transfer model of EAE using a very well defined T cell for inducing EAE. It is important to note that when working with these mice, a very clean mouse facility is absolutely required, as these mice develop a high incidence of spontaneous EAE in conventional animal facilities. It should also be noted that several investigators have used the Vβ8 T cell receptor transgenic mouse for EAE studies and this mouse is also available through the Jackson Laboratory (Ratts et al., 1999). This mouse does not develop spontaneous EAE, but can easily be induced to develop active EAE by immunization with MBP Ac1-11 peptide emulsified in CFA. In the author's experience, this protocol induces 100% incidence of disease and does not require PT in the induction protocol.

When testing genetically manipulated mice, the choice of EAE model is most likely going to be influenced by the genetic background of the transgenic or mutant mouse strain. The susceptibility of many mouse strains to MBP and PLP is shown in Table 9.7.1. In addition, many mutant strains are available on the 129 or B6 background, and in some cases, (B6 × 129) F1 mice. B6 mice and (B6 × 129) F1 mice are resistant to the induction of MBP-induced EAE, but the 129 mice are susceptible to this antigen for both active and passive disease. B6 mice are susceptible to EAE induction with myelin oligodendrocyte

glycoprotein (MOG) or the MOG peptide 35-55 (see Table 9.7.2). Most reports using MOG in the B6 strain employed the active EAE model and the 35-55 peptide.

For testing the therapeutic potential of a reagent in the EAE model, the chronic, relapsing forms of disease are most advantageous because of the relevance of being able to test the therapeutic reagent in the setting of established or ongoing disease. The SJL mouse for relapsing disease has been most widely used for this purpose. Adoptively transferred EAE using MBP as the inducing antigen with either SJL or (PL × SJL) F1 mice produces a chronic, relapsing model that should be quite reproducible within a particular laboratory. For many laboratories, the active EAE model in the SJL mouse with PLP peptide 139-151 is of greatest utility because it produces a relapsing-remitting disease following a single immunization and is technically much simpler than the adoptive transfer model.

Table 9.7.1 Susceptibility of Inbred Strains of Mice to Actively Induced EAE[a]

Strain	MBP[b]	PLP[b]	MOG[b]
B6	No	Yes, but very mild	Yes
SJL/J	Yes	Yes	Yes
DBA/1	Yes, mild	Yes, but very mild	Unknown
PL/J	Yes	Yes	Unknown
C3H	Yes	Yes, but mild	Unknown
Balb/c	No	Yes (depends on substrain)	Unknown
B10	No	Unknown	Unknown
B10.PL	Yes	Yes	Unknown
B10.A	Yes	Unknown	Unknown
B10.RIII	Yes	Unknown	Unknown
B10.S	No	No	No
AKR	Yes	Yes, but mild	Unknown
129	Yes	Unknown	Yes

[a]Inbred mouse strains can be obtained from the Jackson Laboratory, Taconic Farms, Inc., or Harlan Sprague Dawley Inc.
[b]Abbreviations: MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein.

Table 9.7.2 Myelin Peptides Used To Induce EAE

Peptide[a]	Predominant mouse strains when used	Encephalitogenic sequence
MBP Ac1-11	PL/J, B10.PL	ASQKRPSQRSK
MBP 89-101	SJL	FKNIVTPRTPPP
PLP 139-151	SJL	HSLGKWLGHPDKF
MOG 35-55	B6	MEVGWYRSPFSRVVHLYRNGK

[a]These peptides are usually custom-synthesized. The author has had success using peptides synthesized by CS Bio.

BASIC PROTOCOL

ACTIVE INDUCTION OF EAE IN MICE

In this protocol, susceptible strains of mice are immunized with myelin antigen or peptide emulsified in complete Freund's adjuvant (CFA). Mice are also usually administered pertussis toxin (PT) both on the day of immunization and 48 hr later. Depending on the strain of mouse and the antigen used, signs of EAE will become apparent 10 to 15 days after the initial immunization.

Materials

Myelin antigen (MBP, PLP, MOG or other peptide; see Table 9.7.2). Peptides can be custom synthesized or obtained from a commercial source such as CS Bio
PBS (*APPENDIX 2A*)
Complete Freund's adjuvant (CFA; Difco)
Female mice from EAE susceptible strain (see Table 9.7.1), group housed 8 to 12 weeks of age
Methoxyflurane (anesthesia)
Pertussis toxin (PT; List Biological Laboratories)

Omni Mixer (Omni International, Popper & Sons; can also use 2- or 5-ml Micro-mate interchangeable glass syringes connected by a 7/8-in. stainless steel, 18-G micro-emulsifying needle)
1-ml tuberculin syringes
25-G needles
Bell jar
Electric hair clippers
Animal balance (for weighing mice), accurate to 0.1 g

NOTE: Female mice are generally used because they can be group housed without difficulty. Male mice will often fight and need to be housed separately. In SJL mice, females are more susceptible to the development of EAE, but there are mouse strains (e.g., B10.PL) where males are more susceptible.

1. For whole-protein induction of EAE, dissolve either MBP, PLP, or MOG (see Table 9.7.2) in PBS at a concentration of 8 mg/ml.

   If antigenic peptides are to be used for EAE induction, dissolve them at a concentration of 4 mg/ml in PBS.

2a. When using an Omni Mixer: place 1:1 (v/v) antigen and CFA in the small mixing cup and mix for ~3 min at high speed.

   Mix enough antigen in PBS and CFA for two times the number of mice to be immunized. For immunization, use 400 μg/mouse for whole protein or 200 μg/mouse for peptide. Thus, with a 1:1 emulsion of antigen in PBS and CFA, each mouse will receive 100 μl for a total antigenic dose. For example, for 20 mice, use 2 ml of the myelin antigen in PBS and 2 ml of CFA. The emulsion should acquire a thick consistency and white color.

2b. If an Omni Mixer is unavailable: make the emulsion using the 2- or 5-ml Micro-mate glass syringes and 18-G stainless steel micro-emulsifying needle.

   For example, for 20 mice, draw up 2 ml of the myelin antigen in a 5-ml syringe and 2 ml of CFA in another 5-ml syringe. Attach the syringes to a double-ended micro-emulsifying needle and push the plungers back and forth to mix the antigen with the CFA. This process may take several minutes to achieve adequate mixing. As with the Omni Mixer method, a thick, white emulsion should be formed.

3. Transfer the emulsion into 1-ml tuberculin syringes and attach a 25-G needle. Prepare the emulsion just prior to immunization and keep on ice until used.

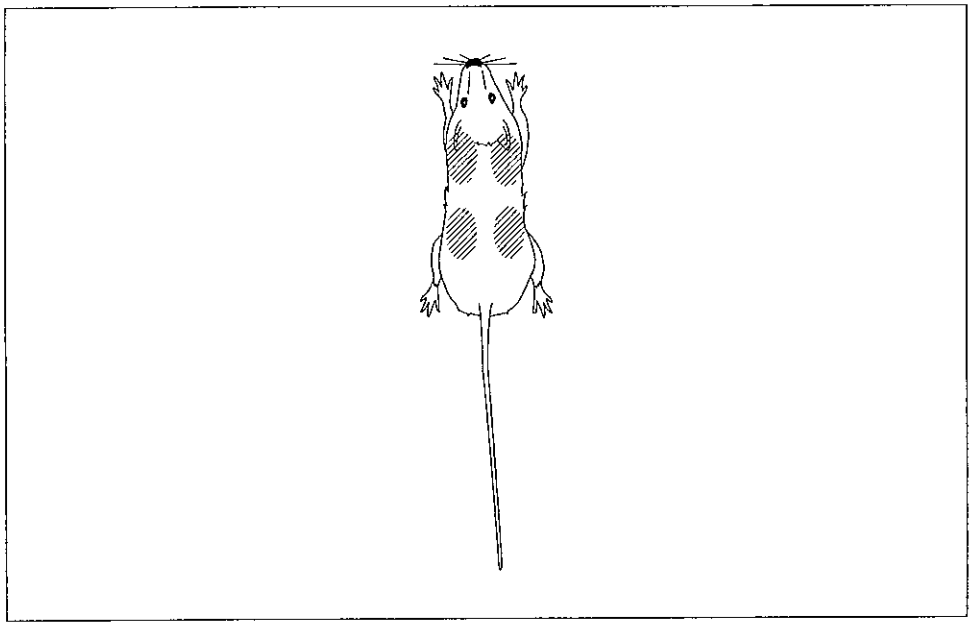

Figure 9.7.1 Anesthetized mice should be shaved in the areas marked by cross-hatching. These areas drain to the axillary and inguinal lymph nodes.

4. Anesthetize mice by placing them in bell jar with methoxyflurane-moistened cotton swabs or paper towels. Anesthetize up to five mice at one time.

   Mice should be anesthetized to the point where they are unresponsive to painful stimuli such as a tail pinch.

5. Shave mice with electric hair clippers over their flanks in preparation for immunization (see Figure 9.7.1). Inject mice subcutaneously in the four shaved areas with ~25 µl of emulsion per site (100 µl/mouse).

   A raised white bleb should be visible just under the skin.

6. Inject mice (i.p. or i.v. via the tail vein) with 200 ng PT dissolved in 200 µl PBS and return mice to their home cage. Repeat 200-ng PT injection 48 hr later.

   Mice do not need to be anesthetized for this step. If using the i.v. route of injection, it is helpful to first dilate the tail vein by warming the tail with a heating lamp or warm water.

7. Weigh animals daily on an animal balance.

   Mice will lose ~10% of their body weight during the acute episode of EAE (usually 4 to 7 days), depending on the severity of the disease.

8. Monitor daily for signs of EAE beginning 1 week after immunization. Grade mice using the following score.

   0: no abnormality
   1: a limp tail
   2: mild hindlimb weakness
   3: severe hindlimb weakness
   4: complete hindlimb paralysis
   5: quadriplegia or premoribund state
   6: death

   For studies involving the use of a therapeutic agent or comparison between several experimental groups, the examiner should be blinded to the therapeutic regimen or experimental protocol. See Figure 9.7.2 for further explanation of scoring system.

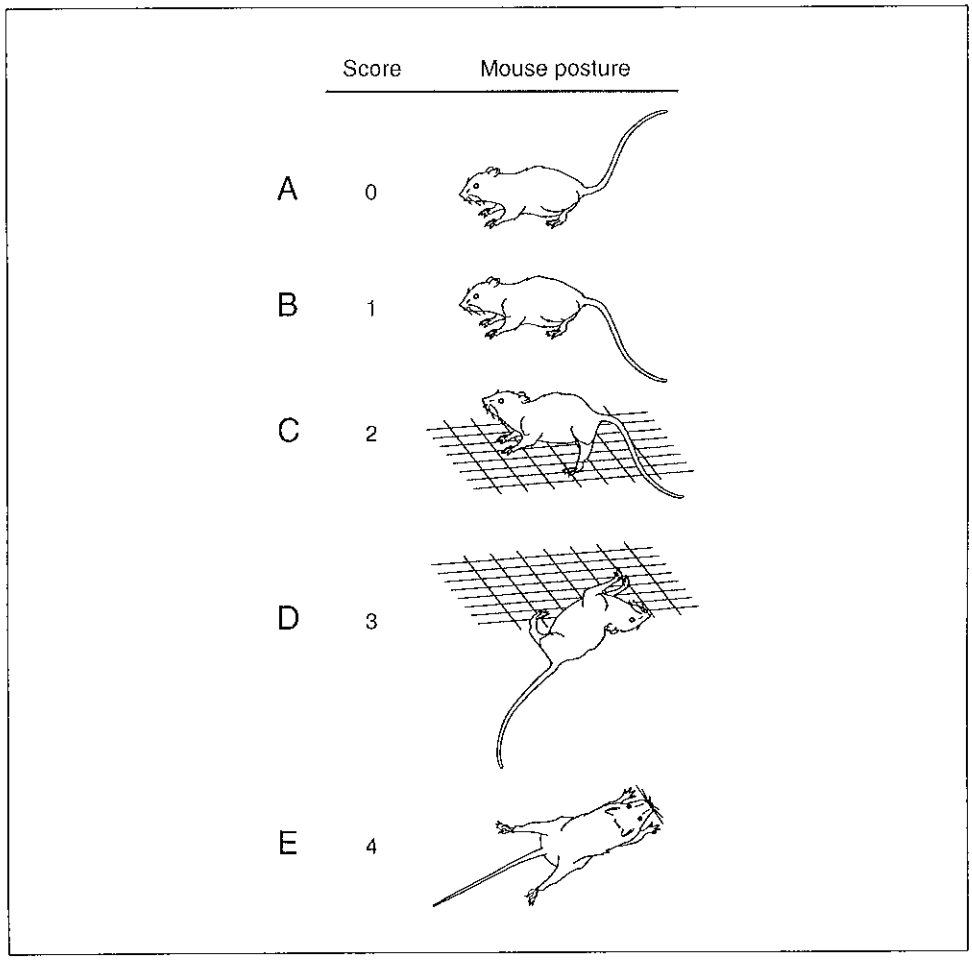

Figure 9.7.2 Clinical scoring of EAE. (**A**) Mice that are removed from a cage will normally respond by having their tail stand straight up. When picking up a mouse by the tail, one can feel that the tail has tone. Such a mouse is normal and represents a clinical score of 0. (**B**) A normal mouse when placed on top of the cage will not misstep between the bars of the cage top. When a mouse has reached a score of 1, the tail no longer stands up on end. When the mouse is picked up by the tail, there is a distinct lack of tone in the tail. However, the mouse walks normally, and when put on the underside of the cage top, can climb to the top without difficulty. (**C**) When a mouse has reached a clinical score of 2, the mouse has a limp tail and shows signs of hindlimb dysfunction. This is most easily detected by placing the mouse on the underside of the cage, where the mouse is able to hang on, but because it does not have the same dexterity of hindlimb movement, it has difficulty climbing to the top side of the cage. When placed on the top side of the cage, the mouse may misstep and the hindlimb foot may protrude between the bars of the cage. (**D**) When the mouse reaches a clinical score of 3, the mouse can no longer hold on to the cage with its hindlimbs when placed on the underside of the cage. However, when ambulating, the mouse still can move the hindlimbs. (**E**) When the mouse reaches a clinical score of 4, the hindlimbs drag behind and are not used by the mouse for movement. A moribund mouse is still alive, but really makes little spontaneous movement and receives a clinical score of 5. These animals are routinely euthanized.

ADOPTIVE TRANSFER OF EAE IN MICE

ALTERNATE PROTOCOL

In passive or adoptively transferred EAE, mice are injected with activated, myelin-specific T cells, thus causing the animals to develop EAE. The activated T cells are generated by immunizing mice with the inducing myelin protein or peptide emulsified in CFA. The draining lymph nodes are removed and the T cells activated again with the inducing antigen in vitro. The activated T cells are then transferred into naïve recipients, which then develop a relapsing and remitting form of EAE.

Additional Materials (also see Basic Protocol)

Complete Hank's balanced salt solution (HBSS; see recipe)
Complete EAE medium (see recipe)
75% ethanol

50-ml conical tubes
Styrofoam dissecting board
Surgical scissors
Jeweler's curved forceps
4.5 × 4.5–cm stainless steel wire mesh screen
60 × 15–mm petri dish, sterile
3-ml plastic syringe
24-well tissue culture plates
Humidified 37°C, 5% CO_2 incubator

Prepare mice

1. Perform Basic Protocol, steps 1 to 5 for active induction of EAE.
2. Prepare HBSS and EAE media prior to lymph node harvest. Put ~25 ml HBSS in a 50-ml conical tube on ice.
3. Sacrifice the mice with an overdose of anesthesia. Pin mouse supine on the dissecting board and spray with 75% ethanol prior to removal of lymph nodes.
4. Using surgical scissors, cut mouse along the midline and cut along all four extremities. Tease back skin and pull it back to expose the axillary and inguinal lymph nodes (see Figure 9.7.3). Using the jeweler's curved forceps, dissect out the inguinal and axillary lymph nodes (6 nodes total). Place each set of nodes into the 50-ml conical tube with the cold HBSS while collecting lymph nodes from all the mice in the particular experiment.

   When exposing the lymph nodes, it is not necessary to open the abdominal wall and expose the internal organs.

Obtain single cell suspensions of lymph nodes

5. Bend a 4.5 × 4.5–cm wire mesh screen at the corners to form a platform and place it in a sterile 60 × 15–mm petri dish. Place 10 to 20 lymph nodes on the screen and press them through the screen with a plunger from a 3-ml plastic syringe. Wash cells remaining on screen with 2 to 4 ml of HBSS and then transfer the media containing the cells to a new 50-ml conical tube. Repeat this until all the lymph nodes have been disrupted into a single cell suspension.
6. Centrifuge the cell suspension for 10 min at 200 × *g* (1000 rpm), 4°C using an Eppendorf A-4-62 swinging bucket rotor. Decant the supernatant and disrupt the pellet by tapping the 50-ml conical tube between the thumb and index finger. Resuspend the cell pellet in 20 ml of HBSS and centrifuge again (this completes the first wash). Repeat the wash, but resuspend the cells from the second wash in complete EAE medium at 4×10^6 cells/ml (see *APPENDIX 3B*). Culture cells with the

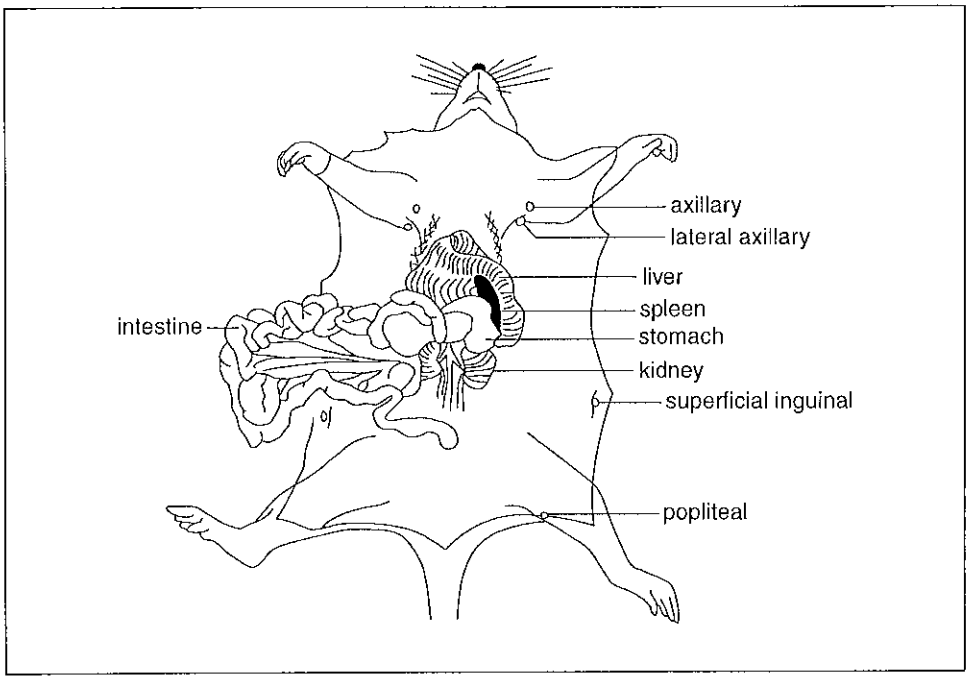

Figure 9.7.3 Adoptive transfer of EAE, lymph node harvest. After mice have been sacrificed, the mouse is pinned on its back and opened with a midline incision. The skin is then peeled from the thorax and also pinned down. The inguinal lymph nodes are present at the junction of two draining veins. The nodes are a dusky color and should not be confused with the CFA/antigen depot, which is white. The axillary nodes are found where the arms join the trunk. One node is very superficial, and may require very little dissection in order to isolate it. The other axillary node is much deeper and requires careful dissection. This node is usually found just medial to the muscles of the upper forelimb.

immunizing myelin antigen in 24-well tissue culture plates (2 ml/well) for 96 hr in a humidified 37°C, 5% CO_2 incubator.

For MBP, PLP, and MOG, a concentration of 25 to 50 μg/ml is optimal for activating the T cells in vitro.

7. Aspirate cells from the 24-well plates, place in 50-ml conical tubes, and centrifuge for 10 min at 200 × *g* (1000 rpm), 4°C. Wash pellets two times with complete HBSS (as in step 6) and count (see *APPENDIX 3B*). In the pilot experiment, inject 3×10^7 cells/mouse i.v. in the tail vein or i.p.

   Animals should develop clinical signs of EAE in 6 or 7 days. Because this is a biological system where numerous factors influence the severity of disease, the number of cells required to cause reproducible EAE may have to be adjusted. If performed well technically, adoptively transferred EAE should result in 100% incidence of disease with all animals showing signs of disease within 2 to 3 days of one another.

8. Monitor animals daily for signs of EAE as described in Basic Protocol, steps 7 and 8 for the active induction of EAE.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see APPENDIX 2A; for suppliers, see SUPPLIERS APPENDIX.

Complete EAE medium

500 ml RPMI without L-glutamine (Life Technologies)
0.5 ml 50 mM 2-mercaptoethanol
5.0 ml 100 mM sodium pyruvate
5.0 ml 10 mM non-essential amino acids
5.0 ml 200 mM L-glutamine
5.0 ml 10,000 U/ml penicillin/10,000 mg/ml streptomycin
6.25 ml 1 M HEPES solution
50.0 ml fetal bovine serum
Total volume 576.75 ml
Store up to 1 month, 4°C

Complete Hank's balanced salt solution (HBSS)

415.0 ml sterile H_2O
50.0 ml 10× HBSS without Ca^{2+} and Mg^{2+} (e.g., Life Technologies)
20.0 ml fetal bovine serum
5.0 ml 1 M HEPES solution
5.0 ml 2.8% sodium bicarbonate solution
5.0 ml 10,000 U/ml penicillin/10,000 mg/ml streptomycin
Total volume 500 ml
Store up to 1 month, 4°C

COMMENTARY

Background Information

During recent years, the understanding of immunological mechanisms involved in demyelination has advanced greatly through the investigation of EAE. This animal model of MS can be induced either by immunization of susceptible animals with components of myelin or adoptive transfer of $CD4^+$ T cells specific for myelin antigens (Zamvil and Steinman, 1990; Martin et al., 1992). The adoptive transfer experiments have clearly established that EAE is a T cell-mediated autoimmune disease. MHC class II background was initially thought to be the most important factor conferring disease susceptibility, but recent studies have shown that other genetic loci are also involved in disease susceptibility (Martin et al., 1992). Another variable in EAE studies has been the antigen used to induce disease. MBP and PLP are the major protein components of myelin and have been most often used as the disease-initiating antigen in EAE. Although EAE produced by immune responses against MBP or PLP is T cell mediated, it has also been shown that addition of antibodies to myelin oligodendrocyte glycoprotein, a minor glycoprotein on the outer surface of the oligodendrocyte, dramatically enhances demyelination in MBP-induced EAE (Linington et al., 1988). It is also thought that antibodies play an important role in MOG-induced EAE (Litzenburger et al., 1998).

Critical Parameters and Troubleshooting

If mice are not developing EAE with the active protocol (see Basic Protocol), then the encephalitogen should be investigated, particularly if it is not a newly synthesized peptide. Potency of the adjuvant can present a problem. CFA from Difco has produced reliable results. Alternatively, CFA can be made in-house by obtaining Mycobacteria H37RA, grinding it with a mortar and pestle, and adding it to IFA. For EAE induction, 30 μg of mycobacteria is required per mouse. When mixing the peptide or protein in CFA, it is important to make sure that a thick, white emulsion is made for immunization. Incomplete mixing of the antigen in CFA can result in suboptimal results.

Before attempting to establish animal models of adoptively transferred EAE (see Alternate Protocol), a proliferation assay should be performed to ensure that the draining lymph node cells are responding well to the immunizing antigen. Proliferative responses can be assessed on antigen-specific lymph node cells by incu-

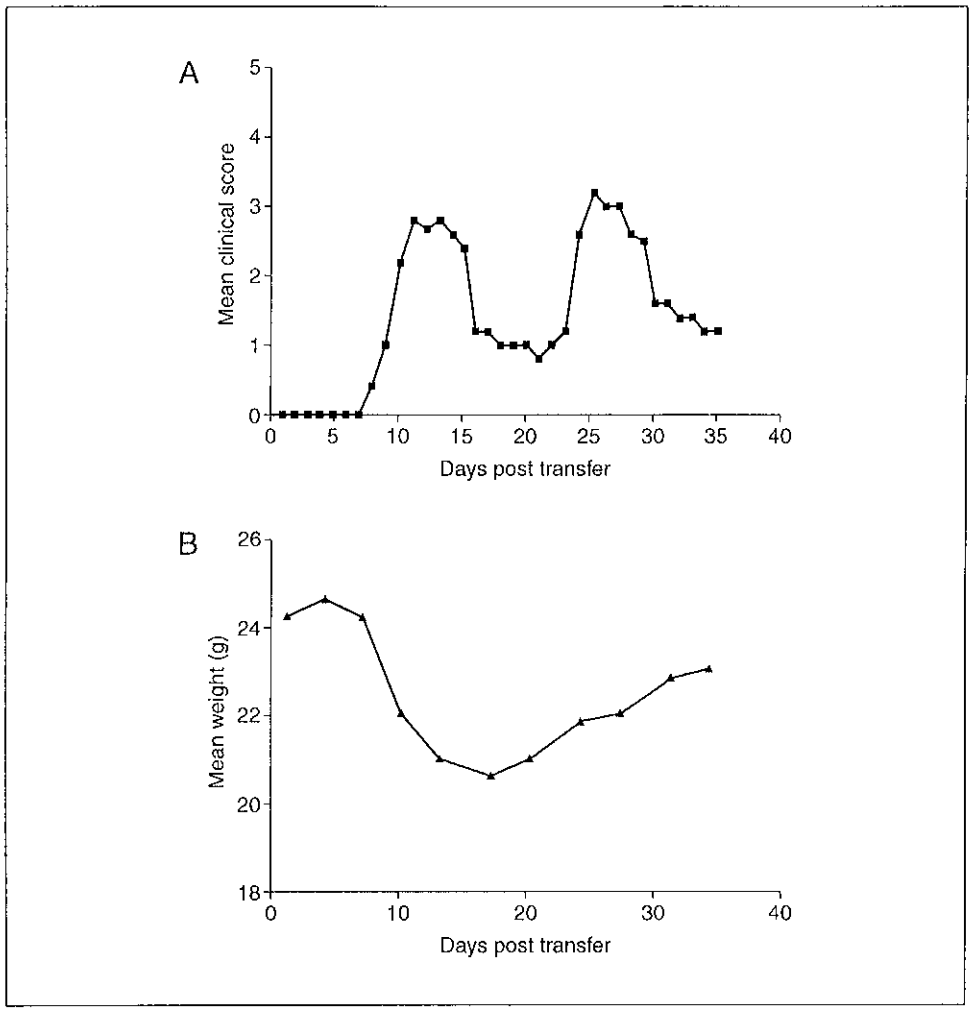

Figure 9.7.4 Activated lymph node cells from MBP/CFA immunized (PI × SJL) F1 mice were transferred into naïve recipients at day 0. (**A**) Mice were monitored daily and a mean clinical score was assigned for each group of five mice. (**B**) Mean weights of the mice from the experiment shown in **A**.

bating cells (2×10^5 cells/well in a 96-well plate) with the antigen, Con A (use 2 to 4 μg/ml as a positive control for T cell stimulation), and medium alone. When initially setting up the model, testing serial dilutions of the antigen will help determine the optimal antigen concentration for expanding encephalitogenic T cells. Typically, proliferation will increase with increasing antigen, but there will be a point where proliferative responses begin to decrease with higher doses, due to activation-induced cell death (Critchfield et al., 1994). Proliferation assays are performed for 96 hr, with the plates being pulsed with 0.5 μCi of [^{3}H]methylthymidine (Amersham Pharmacia Biotech) for the final 16 hr. To quantify thymidine incorporation, cells should be harvested onto glass-fiber filters using a Tomtec 96-well cell harvester and filters assayed for the incorporated thymidine radiolabel using a Betaplate liquid scintillation counter (Wallac).

Anticipated Results

Induction of EAE by active immunization (see Basic Protocol) should be a relatively simple undertaking. However, with the advent of pathogen-free animal facilities, some investigators have found disease induction to be more difficult than anticipated. An additional injection of pertussis toxin 7 days after immunization may be helpful in inducing disease under these circumstances.

Because of the variety of different mouse strains and antigens presently being used in animal models of EAE, not all scenarios have been thoroughly characterized with regard to adoptively transferred EAE (see Alternate Protocol). By far, the majority of studies in adop-

tively transferred EAE have utilized either MBP or PLP peptide 139-151 in the SJL mouse. If utilizing different mouse strains or antigens, culture and transfer conditions may need to be adjusted for optimal results. An example of typical results from an adoptive transfer experiment (see Alternate Protocol) using MBP-specific T cells (3×10^7 cells) in (PL × SJL) F1 mice is shown in Figure 9.7.4.

Time Considerations

The time to establish EAE in the laboratory can be quite variable. However, once a particular model is established, data can be generated rather quickly. The experiments themselves are not very time consuming. Active immunization of mice only takes a couple of hours and this time will decrease with experience. On the other hand, the adoptive transfer protocol (see Alternate Protocol) includes the lymph node harvest and injection steps, which together will take 2 to 3 hr. However, if examining a treatment effect in the chronic, relapsing model, individual experiments may take up to three months to determine long term treatment effects.

Literature Cited

Critchfield, J.M., Racke, M.K., Zúñiga-Pflücker, J.C., Cannella, B., Raine, C.S., Goverman, J., and Lenardo, M.J. 1994. T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. *Science* 263:1139-1143.

Goverman, J., Woods, A., Larson, L., Weiner, L.P., Hood, L., and Zaller, D.M. 1993. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell* 72:551-560.

Linington, C., Bradl, M., Lassman, H., Brunner, C., and Vass, K. 1988. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am. J. Pathol.* 130:443-454.

Litzenburger, T., Fassler, R., Bauer, T., Lassmann, H., Linington, C., Wekerle, H., and Iglesias, A. 1998. B lymphocytes producing demyelinating autoantibodies: Development and function in gene-targeted transgenic mice. *J. Exp. Med.* 188:169-180.

Martin, R., McFarland, H.F., and McFarlin, D.E. 1992. Immunological aspects of demyelinating diseases. *Annu. Rev. Immunol.* 10:153-187.

Racke, M.K., Dhib-Jalbut, S., Cannella, B., Albert, P.S., Raine, C.S., and McFarlin, D.E. 1992. Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor-beta1. *J. Immunol.* 154:2959-2968.

Ratts, R.B., Arredondo, L.R., Bittner, P., Perrin, P.J., Lovett-Racke, A.E., and Racke, M.K. 1999. The role of CTLA-4 in tolerance induction and T cell differentiation in experimental autoimmune encephalomyelitis: i.p. antigen administration. *Int. Immunol.* 11:1881-1888.

Zamvil, S.S., and Steinman, L. 1990. The lymphocyte in experimental allergic encephalomyelitis. *Annu. Rev. Immunol.* 8:579-621.

Contributed by Michael K. Racke
University of Texas
Southwestern Medical Center at Dallas
Dallas, Texas

SIGMA-ALDRICH

Material Safety Data Sheet

Version 3.1
Revision Date 06/18/2009
Print Date 01/27/2010

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Pertussis toxin, from *Bordetella pertussis*
Product Number	:	P7208
Brand	:	Sigma
Company	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA
Telephone	:	+19058299500
Fax	:	+19058299292
Emergency Phone #	:	800-424-9300

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Islet Activating Protein
IAP
Pertussigen
Histamine-sensitizing factor

CAS-No.	EC-No.	Index-No.	Concentration
Pertussis toxin from Bordetella pertussis			
70323-44-3	-	-	-

3. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Pancreas.

WHMIS Classification

D1A Very Toxic Material Causing Immediate and
D1B Serious Toxic Effects

Highly toxic by inhalation
Toxic by ingestion
Toxic by skin absorption

HMIS Classification

Health Hazard:	4
Chronic Health Hazard:	*
Flammability:	0
Physical hazards:	0

Potential Health Effects

Inhalation May be fatal if inhaled. May cause respiratory tract irritation.

Skin	Toxic if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	Toxic if swallowed.

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Flammable properties

Flash point	no data available
Ignition temperature	no data available

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods for cleaning up
Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves.

Eye protection
Face shield and safety glasses

Skin and body protection
Choose body protection according to the amount and concentration of the dangerous substance at the work place.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder, lyophilized

Safety data

pH	no data available
Melting point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Storage stability
Stable under recommended storage conditions.

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

LD50 Intravenous - rat - 0.114 mg/kg
Remarks: Sense Organs and Special Senses (Nose, Eye, Ear, and Taste):Eye:Lacrimation. Behavioral:Change in motor activity (specific assay). Nutritional and Gross Metabolic:Weight loss or decreased weight gain.

Irritation and corrosion

no data available

Sensitisation

no data available

Chronic exposure

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Signs and Symptoms of Exposure

Potentially neurotoxic.

Potential Health Effects

Inhalation	May be fatal if inhaled. May cause respiratory tract irritation.
Skin	Toxic if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	Toxic if swallowed.
Target Organs	Pancreas.,

Additional Information
RTECS: XW5883750

12. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)

no data available

Ecotoxicity effects

no data available

Further information on ecology

no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Pertussis toxin from Bordetella pertussis)
Marine pollutant:

Poison Inhalation Hazard: No

IMDG
UN-Number: 3462 Class: 6.1 Packing group: I EMS-No: F-A, S-A
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (Pertussis toxin from Bordetella pertussis)
Marine pollutant: Marine pollutant

IATA
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid n.o.s. (Pertussis toxin from Bordetella pertussis)

15. REGULATORY INFORMATION

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
Pertussis toxin from Bordetella pertussis	70323-44-3

WHMIS Classification

D1A D1B	Very Toxic Material Causing Immediate and Serious Toxic Effects	Highly toxic by inhalation Toxic by ingestion Toxic by skin absorption

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

B10-uuu-uuuu

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Dr. Bhagirath Singh
SIGNATURE Bhagirath Singh
DEPARTMENT Microbiology + Immunology
ADDRESS Dental Sciences Bldg.
PHONE NUMBER 661-3483
EMAIL bsingh@uwo.ca

Location of experimental work to be carried out: Building(s) SDRI Room(s) 224, 225, 226, 227.
*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S):
Various

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES CHIR

Names of all personnel working under Principal Investigators supervision in this location:
i) Hui-Yu Qin
ii) Edwin Lee-Chan
iii) Olga Krougly
iv) Enayat Nikoopour
v) Jordan Schwartz
vi) Katrina Huszarik

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☒ YES ☐ NO
If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?
BCG: Attenuated strain of Mycobacterium Bovis	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	0.2 ml.
E.coli OH5A	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	500 mls.
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	

1.3 For above named organism(s) or biological agent(s) circle (HC) or CFIA Containment Level required. 1 (2) 3

1.4 Source of microrganism(s) or biological agent(s)? E.coli - Gibco/BRL
BCG - Sanofi Pasteur

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (ie. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	☒ Yes ☐ No	Blood
Rodent T-cells Dendritic-cells	☒ Yes ☐ No	Liver, Kidney, Lymph Nodes Spleen, Pancreas, Bone Marrow
Non-human primate	☐ Yes ☐ No	
Other (specify)		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	☒ Yes ☐ No	U937	ATCC
Rodent	☒ Yes ☐ No	PU5, NIT	ATCC
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

2.4 For above named cell types(s) circle (HC) or CFIA containment level required 1 (2) 3

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☒YES ☐NO
If no, please proceed to Section 4.0

3.2 Indicate if the following will be used in the laboratory

- Human blood (whole) or other bodily fluids ☒YES ☐NO If YES, Specify Whole blood, Synovial Fluid
- Human blood (fraction) or other bodily fluids ☒YES ☐NO If YES, Specify Serum, Cells
- Human organs (unpreserved) ☐YES ☒NO If YES, Specify _____
- Human tissues (unpreserved) ☒YES ☐NO If YES, Specify Synovial Tissue

3.3 Is human source known to be infected with and infectious agent ☐YES ☒NO
If YES , please name infectious agent _____

3.4 For above named materials circle (HC) or CFIA containment level required. 1 (2) 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents or cells described in Sections 1.0 and 2.0 ? ☒YES ☐NO
If no, please proceed to Section 5.0

4.2 Will genetic sequences from the following be involved:

- HIV ☐YES ☒NO
if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens ☐YES ☒NO
if YES specify_____
- Other human or animal pathogen and or their toxins ☐YES ☒NO
if YES specify_____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen ☒YES ☐NO If YES specify NIT cells.
- Known oncogenes ☐YES ☒NO If YES specify_____

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction ☐YES ☒NO
If YES name virus_____.

4.5 List specific vector(s) to be used:_____

4.6 Will virus be replication defective ☐YES ☒NO

4.7 Will virus be infectious to humans or animals ☐YES ☒NO

4.8 Will this be expected to increase the Containment Level required ☐YES ☒NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? ☐ YES ☒ NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? ☐ YES ☐ NO

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? ☐ YES ☐ NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? ☒ YES ☐ NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used mice, Rabbit, rats

6.3 AUS protocol # 2004-008-01, 2004-007-01

6.4 If using murine cell lines, have they been tested for murine pathogens? ☒ YES ☐ NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other bodily fluids including blood be used:

- Pound source dogs ☐ YES ☒ NO
- Pound source cats ☐ YES ☒ NO
- Sheep or goats ☐ YES ☒ NO
- Non- Human Primates ☐ YES ☒ NO If YES specify species _____
- Wild caught animals ☐ YES ☒ NO If YES specify species _____
  colony # _____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? ☒ YES ☐ NO
If no, please proceed to Section 9.0

8.2 If YES, please name the toxin Staphylococcal Enterotoxin B.

8.3 What is the LD_{50} (specify species) of the toxin 0.02 mcg/kg. - from Sigma (Canada)

Biosecurity

* toxin must be kept in a locked cupboard/fridge.
* no more than 5 mg at any time in lab

9.0 Import Requirements

9.1 Will the agent be imported? ☐ YES ☒ NO
If no, please proceed to Section 10.0
If yes, country of origin_____

9.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

9.3 Has an import permit been obtained from CFIA for animal pathogens? ☐ YES ☐ NO

9.4 Has the import permit been sent to OHS? ☐ YES ☐ NO
If yes, Permit # _____

10.0 Training Requirements for Personnel named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE Bhagwat Singh

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest (HC) or CFIA Containment Level required. 1 (2) 3

11.2 Has the facility been certified by OHS for this level of containment? ☒ YES ☐ NO

11.3 If yes, please give the date and permit number: June 20, 2006 BIO-UWO-0066 .

12.0 Approvals

UWO Biohazard Subcommittee

Signature G.M. Gilbert Date 24 Aug. 07

Safety Officer for Institution where experiments will take place

Signature J Stanley Date Aug 24/07

Safety Officer for University of Western Ontario (if different than above)

Signature_____ Date_____

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*