



Dave Morens

Memorandum

Date September 28, 1981

From Steven J. McDougal, M.D.
Medical Officer

Subject Protocol

To Bruce L. Evatt, M. C.
Director, Host Factors Division

Enclosed is the Protocol you asked for. I am sending copies to the people mentioned below but will leave forwarding the copy to Dowdle to you.

Sincerely yours,

Steven J. McDougal, M.C.

cc: Dave Gordon, M.D.
Tom Spira, M.D.
Alex Kelter, M.D. (3-108D)
Dave Morens (will pick up)
Renate Kimbrough, M.D. (31 Chamblee)

DARROW 10/5/81
KSOI

INHALATION STUDY PROTOCOL

Summary

I. Facilities:

2 separate animal rooms, 1 for MCMV infected mice
and the other for uninfected mice

5 inhalation chambers

Precautions for explosive inhalants

II. Animals:

380 6-8 week old ICR mice

60 litters ICR mice

III. Personnel:

1 GS-9; animal handling, exposure, chemicals, etc.

1 GS-9; injections, bleeding, autopsies, etc.

Protocol: Amyl nitrite inhalation: effect on immunocompetence in mice

I. Synopsis of proposal: The impetus for this study is the observation that Kaposi's Sarcoma and opportunistic infections in homosexual men ~~has~~ ^{have} an association with amyl nitrite inhalation. We propose to use an exposure scheme and a set of functional immune measurements which, if amyl nitrite has a major effect on immune competence, ~~we~~ ^{we} will ~~be~~ ^{be} detect ~~it~~. Subtle effects may be missed ~~by this protocol~~ due to inappropriate exposure or insensitivity or irrelevance of the assays; however, as a first approach, a screen for major defects is a more sensible and efficient approach. This proposal was prepared on 24 hours notice, and is to be considered a tentative offering which may be subsequently amended, expanded, or contracted.

II. Materials, Methods, and Preliminary Experiments

Six to 8 week old

age?
Mice: A ICR, specific pathogen free mice are bred and readily available from the CDC Lawrenceville facility. This is an outbred strain and as opposed to inbreds less likely to have idiosyncratic reactivities. If a defect is found which requires further more elaborate manipulation, i.e. cell transfers, the phenomenon will have to be quickly confirmed in an inbred strain.

will be used
Group sizes: Twenty exposed mice and twenty age ~~plus~~ ^{sex} matched controls for each exposure group. Ten exposed plus ten control mice will be tested for antibody response, lymphocyte phenotype, and hematological parameters. The other half (ten exposed plus ten control) will be tested for delayed type hypersensitivity responses and pathological post mortem exam.

Exposure of suckling mice will require the generation of thirty litters to obtain ten pairs of age matched litters (± one day) for exposure and non-exposure.

Preliminary Experiments:

Immune parameters: The immunological methodologies are extant and have been reasonably reproducible. They will be repeated and practiced on a small group of animals prior to the actual experiment. Optimal doses and schedules are known.

Exposure parameters: Since the toxic dose is not known, we will have to pretitrate the exposure. We wish to use a dose ~~which~~ ^{that} does not cause generalized toxicity, which we will assess by body weight. The highest dose and schedule which does not result in greater than 5% loss of body weight will be used. General considerations regarding exposure are the following:

1. Mice should not take up greater than 5% (ideally less than 1%) of the volume of the inhalation chamber.

Group	Route	Notes
IP	IP	10-2000
IP	IP	5-1000
CC	CC	4-1000

*mice now - 3-4 wks later no detectable ...
PHA response delay ...
14 day ...*

2. The airflow should be sufficient such that no accumulation of ammonia (from excrement) occurs. Although amyl nitrite does not react with ammonia in air, it is not known what the effect of ammonia is on immune competence.
3. Specifics of airflow are not yet known until we know what inhalation chambers are to be used (one possibility is to buy larger (100 liter) aquariums and adapt them for flow studies; the other possibility is to use NIOSH equipment).
4. Amyl nitrite is explosive. Proper ventilation and precautions are required.
5. Remove food and transfer to separate cages for exposure to eliminate adsorption of amyl nitrite to items in environment when animals are not exposed.
6. Amyl nitrite will be delivered by bubbling through air with subsequent mixing with normal air to give the desired concentrations. Pumps, flow meters, and a calibration assay will be needed for this.
7. Calculated doses:

mice tidal volume = 0.1 ml.
respiratory rate = 250/min

amyl nitrite concentration	100 ppm	10
	478 mg/m ³ air	48
assumed absorption	50%	50
dose (6 hour exposure) per mouse	2.2 mg/mouse	.22
dose (6 hour exposure) per kg	6.6 gms/kg	.66

8. Initial screening exposure:

Group 1 10 ppm x 6 hours x 5 days/wk x 3 wks
Group 2 100 ppm x 6 hours x 5 days/wk x 3 wks

1. follow body weight
2. select dose which does not result in significant weight loss

Clinical and lab measurements to be obtained in exposed verses control groups:

1. Body weight
2. Survival (hopefully 100%)
3. Hematological parameters WBC, Hct, methemoglobin
4. Primary anti sheep erythrocyte (SRBC) antibody response (requires an intact macrophage, T, B, and plasma cell system for normal response).

need to prevent formation of nitrosamines which may occur noted by exposure to organic nitrates

Experiment C:

1. Expose 10 litters (10 control litters).
2. Follow for clinical evidence of runt disease.
3. Sacrifice as indicated by clinical observations (or at 21 days) for histopathological examination and spleen and thymus weights.

IV. Final notes

Unfortunately, we have not included an assay of H-2 recognition or restricted cytolytic capacity (MLC, GVH assay, tumor or skin graft rejection). This should ultimately be done, however, these assays require inbreds, can be quite cumbersome, will require preliminary experimentation for optimizing the responses. Therefore, I think they should reasonably be deferred until this phase I protocol is complete so that hopefully we will be able to confine the experimentation to a small number (? one) exposure protocol.

We have purposefully omitted from these screening assays mitogen or antigen proliferative assays because 1) their functional significance is questionable 2) the inherent variability of these assays require a massive defect to make any statistical sense out of the data.

The division of labor is as follows:

Dr. Kimbrough and Group: Predetermination of dose, decision regarding the number of exposure groups, set up of inhalation chambers, and exposure of mice.

Dr. McDougal and Group: Injections and assays. Mice will be housed at Chamblee. McDougal's technician will travel to Chamblee for injections. On day 0 the DTH and its control group will have assays read at Chamblee and the mice will be left for histological exam. The SRBC antibody group and controls will be transferred on day 0 to Clifton Road for assay.

Regarding inhalation chambers, either chambers rigged from aquariums will be made or inhalation chambers will be obtained from Cincinatti (NIOSH). It is not feasible to do the inhalation in Cincinatti and ship the mice here for study. (Transport by mail is an untoward stress on mice and an unwanted variable).

Conceivably the study could be done in Cincinatti but they would have to send someone here for training. I would not like to spare personnel to go to Cincinatti with their reagents and materials for three separate weeks or for the inevitable subsequent experimentation required.

Role of CMV virus:

The foregoing protocol does not take into account the possible role of CMV or other opportunistic infection synergizing to produce an immune deficit.

need to discuss this

The CMV mouse infection (latent and acute) models are not extant methodologies at the center and would require establishing preliminary parameters before experimentation. It is likely that the 12 week protocol enumerated above could be completed prior to the time the CMV model is established and, if so, no time would be lost plus, based on the above experiments, we might be able to confine the experimentation to one exposure protocol. If so, the groups to be examined would be as follows:

Group 1, Control; Group 2, amyl nitrite exposed; Group 3, CMV infected; Group 4, CMV infected and amyl nitrite exposed. Two separate animal rooms and exposure rooms will be required for this study.

V. Equipment Mouse Requirements

Exp A Two inhalation chambers
20 mice

Exp B Inhalation chamber(2) (capacity 60 mice)
120 mice

Exp C Inhalation chamber (capacity 10 litters)
30 litters

Exp D 2 separate inhalation chambers
2 separate animal rooms
240 mice

} can be done
simultaneously

Additional info:

Plan to run above experiments in parallel with MCMV infected 6-8 week old ICR mice initially exposed to nitrites on days 2, 10, and 90 after exposure*. Also plan to add additional positive control groups of mice exposed to nitrosamines, with and without CMV infection.

* ^{Lymphocyte} Murine PHA stimulation increases 0-5 days after MCMV infection, is depressed 5-15 days after infection, then rises to peak at about 90 days post infection.