THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

TRANSFER OF IMMUNITY TO *HYMENOLEPIS NANA* IN MICE WITH SPLEEN CELLS OR SPLEEN CELL FRACTIONS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

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Oklahoma City, Oklahoma

1970

TRANSFER OF IMMUNITY TO *HYMENOLEPIS NANA* IN MICE

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ACKNOWLEDGMENT

I wish to extend my heartfelt gratitude and deepest appreciation to Dr. Wallace Friedberg, director of this study, for his continued interest, advice, and immense help throughout the investigation and in the preparation of the manuscript.

I am deeply indebted to my major professor Dr. Michael H. Ivey for his encouragement, guidance, help and support during my academic program. I am grateful to Dr. Philip E. Smith for his inspiration and advice, and the continued help he has given me since my arrival in this country.

My sincere appreciation is extended to Dr. Gilbert A. Castro and Dr. Robert A. Patnode who so kindly served on my Advisory and Reading Committee.

The technical assistance of Miss B. R. Neas, Mr. D. N. Faulkner, and Mrs. M. H. Friedberg is greatly appreciated. I also wish to thank the Civil Aeromedical Institute of the Federal Aviation Administration for making available the necessary facilities for this research.

Finally, I wish to thank my wife. Her willingness to endure many hardships made my graduate studies possible.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The dwarf tapeworm, *Hymenolepis nana*, is the only cestode known in which a direct cycle of propagation is possible. In the direct cycle, *H. nana* eggs excreted with the feces of an infected animal are ingested by a suitable host, the oncospheres are liberated into the lumen of the stomach or intestine, burrow into the tissue of the upper small intestine, and develop into cysticercoids in the villi; in about 96 to 105 hours, the larvae break out of the villi and complete their adult development in the lower small intestine (Woodland, 1924). An indirect cycle of development involving an intermediate host was first demonstrated by Bacigalupo (1928) who found that fleas, grain beetles, and mealworm beetles, previously fed *H. nana* eggs, harbored cysticercoids which were infectious to a definitive host. Unlike the direct cycle, in the indirect cycle there is no tissue phase of parasite development in the definitive host. Experimentally, if *H. nana* eggs are administered by intraperitoneal (Astafiev, 1966), or by subcutaneous or intramuscular injection (DiConza, 1967), the parasite will develop only to the cysticercoid stage and soon die.

Grassi (1887), and later Joyeux (1920), reported that one infection in rats with eggs of H. nana made a second infection impossible. Additional evidence of a resistance to reinfection conferred through infection with the egg stage was reported by Brumpt (1933), Shorb (1933), Hunninen (1935a), and Hearin (1941); see also review by Larsh (1951). In contrast, active immunity could not be induced by adult worms which were transplanted to the intestine of normal mice by a surgical procedure (Hearin, 1941). The absence of a tissue phase in the indirect life cycle of the dwarf tapeworm is generally accepted as the explanation for the lack of immunity in the final host (Heyneman, 1963). Acquired immunity to H. nana is effective against both egg and cysticercoid challenge (Heyneman, 1963).

The question of the mechanism of acquired immunity to H. nana has evoked considerable conjecture and poses many unsettled questions. Of primary interest is the involvement of humoral and cellular defense factors in immunity. Also, the relationship between factors involved in immunity and those operable during the so-called crowding effect invites speculation. A crowding effect was reported for both the cysticercoid (Hunninen, 1935a) and adult stages of development (Woodland, 1924; Shorb, 1933; Hunninen, 1935a; and Read, 1951) as it was observed that the percentage of cysticercoids that developed from eggs decreased with increase in the number of challenge eggs and that the average worm length was inversely proportional to the number present.

Direct evidence of a humoral factor being involved in immunity was reported by Hearin (1941) who induced partial resistance to H. nana by injecting mice with serum from immunized donors. Complement fixing,

agglutinating, and precipitating antibodies have been demonstrated in the sera of H. nana-infected mice (Larsh, 1943). A low level of passive immunity can be transferred in mice from mother to nursing-young via the milk (Larsh, 1942), which suggests the possible involvement of secretory IgA in immunity to H. nana. Weinmann (1966) attempted passive transfer of immunity with serum but was successful in only five of eleven experiments. Weinmann noted also that there was no correlation between protective potency of the serum and complement-fixing antibody titer. Heyneman and Welsh (1959) reported that antiserum from rabbits immunized by injection of whole worm homogenate adversely affected all stages of H. nana development in vitro. In a similar experiment by Weinmann (1966) serum from immunized mice was not effective.

From a study of initial infection with eggs of H. nana and onset of a protective host reaction against a challenge with the same parasite, Heyneman (1962) postulated that local antibody production is an important host response to tissue invasion by H. nana and other helminths. Weinmann (1966) observed numerous plasmocytes in the intestinal wall after challenge infection in mice immunized to H. nana. In examining local antibody production in mice, Coleman et al., (1965) surgically sequestered and irradiated the small intestine and then implanted H. nana adults. They were unable to detect any inhibition of hemagglutinating antibody titer unless the rest of the animal's body was shielded. This suggested that, at least for the adult worm, antibody is produced at sites other than the intestine or in addition to sites along the intestine.

Bailey (1951) observed cellular infiltration in the tissue around H. nana larvae on initial infection and noted that challenge oncospheres

encountered a heightened tissue reaction. On the basis of parasite counts in tissue sections from mice killed 18 to 50 hours after egg challenge, Bailey concluded that very few oncospheres from a second infection were able to penetrate the intestinal tissue and those that did were destroyed by an accelerated host tissue response. Recently Friedberg et al. (1969) reinvestigated this problem and observed that equal numbers of oncospheres penetrated the intestinal tissue in immunized and non-immunized mice after egg challenge. However, in the immunized mice development of the parasites was aborted before they reached the cysticercoid stage and they underwent lysis and disappeared.

Work by a number of investigators suggests that delayed-type hypersensitivity (cell-mediated immunity) may have a significant role in acquired immunity to metazoan parasites. Larsh et al. (1964) transferred peritoneal cells removed from donor mice infected with Trichinella spiralis to normal recipients and observed that significantly fewer adult worms could be recovered from these mice than from controls given the same challenge infection. From this observation, Larsh concluded that a delayed hypersensitivity reaction was responsible for the accelerated removal of adult worms that occurs in previously infected animals. Wagland and Dineen (1965) showed that transfer of mesenteric lymph-node cells from guinea pigs immunized to Trichostrongylus colubriformis prevented a primary infection from reaching patency in non-immune recipients of the same inbred strain, whereas a single injection of serum from resistant donors did not have any noticeable effect on the development of primary infection. They suggested that the control of these helminths, in which all stages of infection are confined to the alimentary tract, may be

mediated by a delayed-type hypersensitivity reaction. Miller (1967) reported that dogs injected with lymph-node cells from donors immunized to Ancylostoma caninum resisted a challenge infection better than untreated controls. He noted a typical delayed hypersensitivity skin response (cellular infiltration and induration) to the challenge larva when they penetrated the skin of the recipients. Lang et al. (1967) transferred partial immunity to Fasciola hepatica to non-immunized mice with peritoneal exudate cells from infected donors.

Transfer of immunity to H. nana with cells was first reported by Friedberg et al. (1967). In that study, x-irradiated mice given spleen cells from immunized donors developed significantly fewer parasites on egg challenge than similarly irradiated controls given spleen cells from non-immunized donors. Weinmann (1968) reported that splenectomy of adult mice had no effect on natural or acquired immunity to H. nana.

The ability to transfer immunity to H. nana and other parasites with cells, but not with serum, brings to mind the studies of cellular transfer of delayed-type hypersensitivity to tuberculin reported by Chase (1945).

The role of delayed-type hypersensitivity, either in recovery from infection or in development of specific long lasting immunity, is uncertain. In tuberculosis, within certain limits delayed-type hypersensitivity contributes to effective immunity, but it may also be a disadvantage to the host because of the intense cellular reaction around focal infection (Burrows, 1968). The possible relationship between the delayed-type hypersensitivity reaction in viral and bacterial infection and

immunity was reviewed by Uhr (1966) who concluded that the relationship is not clear.

The transfer of immune capacity by viable cells is referred to as adoptive immunity. Cells from immunized donors are inoculated into recipients of the same species which are incapable of an immune response to antigenic challenge. Leukocytes from various sources and whole blood have been used for transfer (Brownstone et al., 1966). Donor cells may be injected into recipients intravenously or intraperitoneally. Sometimes the cells are placed in millipore diffusion chambers to allow periodic observations of cell morphology or to obtain cytokinetic data (Algire et al., 1954; Capalbo et al., 1964). Donor cells for transfer may be obtained from an animal that has been appropriately immunized or by incubating normal cells with a specific antigen prior to transfer (Harris et al., 1956). Sometimes recipients are x-irradiated. Irradiation of syngeneic recipients results in enhanced antibody synthesis by transplanted, sensitized cells but the mechanism is not understood (Makinodan et al., 1956; Dixon and McConahey, 1963; Celada, 1966). In allogeneic transfer, x-irradiation prevents rejection of donor cells by depressing the host immune capacity (Harris and Harris, 1951; Makinodan et al., 1956).

The effect of x-radiation on immune responses has been studied extensively (Jacobson et al., 1949; Taliaferro and Taliaferro, 1951; Jacobson and Robson, 1952; Talmage, 1955; Dixon, 1957; Ruch and Wolff, 1957; Porter, 1960; and Thorbecke et al., 1964). Immediately after irradiation, there is a rapid reduction in the number of lymphocytes in the peripheral blood (Taylor et al., 1919; Wald et al., 1962) and loss of

capacity for a primary antibody response to antigen. About 30 days are required for the animal to recover (Dixon et al., 1952a).

Acute radiation death, as caused by whole body x-irradiation, is due chiefly to the destruction of the hematopoietic system (Smith and Congdon, 1960). Bone marrow transplantation can result in repopulation of hemopoietic tissue in the irradiated recipients (DeVries and Vos, 1959). Tissues which have proved effective, in addition to bone marrow, include spleen and fetal liver (Micklem and Ford, 1960; Jacobson et al., 1956). Normally, cells administered intravenously are more effective than those given intraperitoneally relative to the number of cells required to give comparable survival of lethally irradiated animals (Smith and Congdon, 1960). However, despite this advantage of the intravenous route, intraperitoneal injection provides a means of administering certain preparations of hematopoietic tissue which may cause death by embolism if they are injected intravenously.

Using cell suspensions transferred to irradiated, syngeneic recipients, Claman and his colleagues concluded recently that stem cells needed to make antibody against certain antigens are present in the spleen and also in thymus-marrow cell combinations. Thymus or marrow cells alone are not immunocompetent, whereas the intact spleen has all the cellular elements necessary for evoking an immune response (Claman et al., 1966a, 1966b; Hayes and Claman, 1970). The addition of bone marrow cells from non-immune donors enhances the immune response of irradiated mice reconstituted with spleen cells from immunized donors (Radovich et al., 1968).

Transfer of spleen cells from donor mice to lethally irradiated syngeneic recipients has been investigated extensively by Makinodan and

his associates (see Makinodan and Albright, 1966). These studies indicated that the amount of antibody produced is related to the number of primed cells transferred and that for a maximum antibody response there is an optimum dose of antigen for immunization of the donors. Their results also suggested that non-primed cells are sensitive to the requirement of the irradiated recipient for hemopoietic cells, whereas primed cells are not (Perkins and Makinodan, 1964). These relationships may be different for different antigens (Sell and Asofsky, 1968).

The transfer of antibody-forming capacity from immunized rabbits to homologous recipients by means of nucleoprotein containing fractions was first reported by Sterzl and Hrubesova (1956). In 1963 Fong et al. reported that cell-mediated immunity to the tubercle bacillus could be induced in normal animals with the ribosomal fraction of histiocytes from immunized rabbits. They established the importance of RNA in the induction of cell-mediated immunity by isolating an active ribosomal RNA fraction which was inactivated by ribonuclease but resisted the action of deoxyribonuclease and trypsin. Sato and Mitsuhashi (1965) reported that normal monocytes became sensitized when they were incubated with the ribosomal fraction of monocytes from mice immunized to Salmonella enteritidis. Friedman (1964a) transferred immunity to Shigella paradysenteriae from immunized to non-immunized rabbits with lymph-node cell fractions. Cell-free homogenate, nuclei, mitochondria, and ribonucleoprotein containing fractions from immune donors were all active in inducing the formation of anti-shigella agglutinating antibody in recipients.

Following the observation by Garvey and Campbell (1957) that RNA-antigen complex acted as a "super" antigen, there has been widespread

interest in the role of subcellular fractions in antibody formation. Evidence that RNA is involved in the initiation of antibody formation by lymphocytes was originally obtained by Fishman and his coworkers (1961, 1963), who observed an increase in antiphage antibody in a culture that contained lymphocytes from non-immune rats and macrophages from rats immunized to T_2 phage. An immunologically active material that contained RNA was extracted from the macrophages. These findings were confirmed later by many workers. Using the Jerne plaque technique, Cohen and Parks (1965), Cohen et al., (1965), Cohen (1967) and Friedman (1964b) observed that RNA-rich preparations or RNA extracted from the spleen of mice immunized with sheep red cells induced some spleen cells from non-immunized mice to form antibody in vitro. Askonas and Rhodes (1965) and Friedman (1965) reported that small amounts of antigen fragments were present in the active RNA extracts prepared by a similar procedure from tissues obtained from immunized mice, rats, and rabbits. Recently, nucleic acid-rich extracts prepared from spleens of mice immunized with a soluble Shigella antigen were used to induce a specific immune response in normal mice (Friedman, 1969) and spleen cells from non-immunized rabbits were shown to convert to antibody plaque-forming cells by incubation with RNA extracts of peritoneal exudate cells or lymph-node cells from immunized rabbits (Bell and Dray, 1969). The nature and mode of action of such nucleic acid-rich extracts is still uncertain and is currently under investigation in many laboratories.

The present investigation was undertaken to consider the various factors that influence adoptive immunity to H. nana in mice, including the route of immunization of the donors and x-irradiation of recipients.

Also, the preparation and testing of spleen cell homogenate and microsomal and total RNA fractions for transfer of immunity were studied.

CHAPTER II

MATERIALS AND METHODS

Vertebrate Hosts

Male C_3BF_1/Cum mice[1] were employed throughout the investigation as donors and recipients, except in one experiment in which the recipients were male CD_1 mice.[2] All mice were maintained on Purina Laboratory Chow[3] and chlorinated water (8 ml Chlorox/40 gallons tap water). Except where otherwise specified, the mice were between 8 to 12 weeks old at the start of each experiment. They were housed in polycarbonate pans (10" x 18-1/2" x 5"), usually in groups of 15 animals each. Following irradiation, the mice were housed individually. Non-infected animals were kept separately from the infected ones.

Intermediate Host

Flour beetles, Tribolium confusum, were kept in a glass jar and maintained on whole wheat flour containing no preservative. The beetles were obtained from the Biology Department at Rice University and maintained in the Radiobiology Laboratory of Aeromedical Research Institute, FAA, since 1964.

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[1]Cumberland View Farms, Clinton, Tennessee.

[2]Charles River Laboratory, Wilmington, Massachusetts.

[3]Ralston Purina Co., St. Louis, Missouri.

Parasite Maintenance

A laboratory strain of Hymenolepis nana var. fraterna was used throughout these studies. The stock infection was maintained in flour beetles. For this purpose, freshly emerged adult beetles were starved for 10 to 15 days prior to infection. The H. nana eggs, or crushed worms on a circle of moistened filter paper, were put into a petri dish with the beetles for consumption. Two days later, whole wheat flour was added. Mature cysticerocoids developed in the beetles in about three weeks. Beetles remain infected with viable cysticercoids for many months. New beetle larvae were screened out at monthly intervals to avoid dilution of infective cultures by subsequent generations.

Infected beetles were placed in Tyrode solution[4] in a petri dish and dissected under a dissecting microscope. The head, elytra, and wings were removed and the abdomen was opened. The readily released cysticercoids were then collected in groups. A dozen cysticercoids contained in 0.2 ml of Tyrode solution were introduced directly into the esophagus of the lightly anesthetized mouse by means of a syringe fitted with an 18-gauge hypodermic needle and a polyethylene tube. H. nana adult worms were harvested from the posterior end of the mouse ileum about 9 to 12 days after the infection.

Preparation of H. nana Eggs

Eggs were obtained by grinding mature and gravid worm proglottids with 5 ml of Tyrode solution in a 15 ml glass Potter Elvehjem homogenizer. The suspension was filtered through a 200-mesh stainless steel screen[5]

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[4]TC Tyrode Solution, DIFCO Lab., Detroit, Michigan.

[5]Small Parts Inc., Miami, Florida.

(pore size 74 μ) to remove all large particles, and the mature eggs were then collected on a 400-mesh screen (pore size 38μ). The egg suspension was mixed with a magnetic stirrer at low speed to achieve a uniform dispersion. Egg counts were made under the low power objective (100 X) of the microscope with a 0.01 ml sample of the egg suspension on a gridded glass slide (80 squares in an area 16 X 16 mm). Three samples were counted and the average was used to calculate the total egg count. The total volume of suspension was adjusted so that each animal received the desired number of eggs in a specified volume of Tyrode solution. In order to determine the number of eggs in the final egg suspension, three additional egg counts were made after adjusting the volume.

Immunization of Mice

Mice were usually given two immunizing egg doses, under light ether anesthesia. The first dose was approximately 5,000 eggs and the second, given 3 weeks later, was approximately 10,000 eggs. Initially, the effect of the route of administration of the eggs was studied. To administer eggs *via* the oral route, use was made of a 1 ml tuberculin syringe with a Luer Lock connector fitted with a blunted 18-gauge hypodermic needle and a polyethylene tube that extended about 25 mm from the end of the needle. The egg suspension was administered far down in the esophagus. Eggs given by the intraperitoneal route were administered with a 1 ml syringe fitted with a 24-gauge needle. Some mice received eggs by both the oral and intraperitoneal routes. These animals were given a total of 10,000 eggs initially and 20,000 eggs three weeks later. Eggs given by the intravenous route were injected into a tail vein through a 27-gauge needle.

X-Irradiation

X-rays were produced by a Keleket constant potential therapy machine[6] operated at 250 KV and 15 mA. Other radiation factors were: inherent filtration equivalent to 3.5 mm Al, added filtration 0.25 mm Cu and 1.0 mm Al, half-value layer 1.18 mm Cu, target to mouse midline 82 cm. In all experiments, the irradiated mice were given a single whole-body exposure of 950 R at a dose rate of 75 to 78 R/minute. During irradiation the mice were confined in individual perforated Plexiglas containers on a revolving Plexiglas turntable (18 rev/min). The exposure dose-rate was measured and checked with a 250 R Victoreen Condenser R-Meter[7] placed in the center position of an empty Plexiglas container. Mice were irradiated in a group of ten.

In most of the experiments in which cell recipients were irradiated (Tables 4, 5, 6, 7, 9, 10, 11, and 14), a group of radiation control mice (no cells given) was included to confirm that a lethal dose of radiation was administered. The radiation control mice all died within 12 days after irradiation.

Collection of Mouse Bone Marrow

The femurs were removed and stored overnight in the refrigerator at 2 C prior to the preparation of the cell suspension and the marrow was injected within 1-1/2 hours after the cell preparation. To prepare the cell suspension, the epiphysis was cut off and the bone marrow "plug" was flushed out by forcing Tyrode solution through the shaft by use of a

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[6]Standard X-Ray Co., Chicago, Illinois.

[7]Victoreen Instrument Co., Cleveland, Ohio.

syringe and 23-gauge needle. Marrow clumps were broken up by drawing them through the needle several times. The total number of nucleated cells was counted in a Bright Line Hemocytometer.[8] A sample of marrow cell suspension was drawn into a red blood cell pipette to the 5 mark, and brought to the 101 mark by the further addition of 1% acetic acid colored with methylene blue (2 drops of 1% methylene blue diluted to 100 ml with 1% acetic acid in water). The pipette was agitated for one minute on a Microstir.[9] The first four drops were expelled from the pipette. A Levy Double Neubauer Chamber[8] was cleaned with 95% ethanol. Both sides of the hemocytometer chamber counting slide were filled from the remaining mixture. Two counts were made for each sample and the counts were averaged. The cell suspension was then adjusted to contain 4×10^7 cells per ml.

Preparation of Spleen Cell Suspension

Mice were killed by cervical dislocation and the spleens were removed. The cell suspension was prepared with groups of seven spleens, each of which was cut into 3 pieces and placed in a 15 ml glass Potter Elvehjem tissue grinder together with 10 ml of Tyrode solution containing 5% normal mouse serum. Gentle pressure was applied to the plunger of the tissue grinder to disrupt the tissue fragments into a cloudy suspension. The suspension was then filtered through a 100-mesh stainless steel wire screen to remove particles of tissue. Total white cells were counted and the cell concentration was adjusted by the same method as that used with

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[8]American Optical Co., Buffalo, N. Y.

[9]Burton Manufacturing Co., Van Nuys, Los Angeles, California.

the bone marrow. About 1 X 10^8 cells, in a volume of 1 ml, were normally given to lethally x-irradiated mice by tail vein injection.

Preparation of Spleen Homogenate

The procedure used to prepare the spleen homogenate was essentially the same as that used for the spleen cell suspension except that the spleen fragments were subjected to freezing (dry ice and ethanol) and thawing (tap water) before homogenation in a Potter Elvehjem tissue grinder.

Preparation of Spleen Microsome Fraction

Spleen homogenate, prepared as described above, was centrifuged at 14,500 X g for 15 minutes at 4 C in a Servall RC-2 refrigerated centrifuge.[10] The supernatant was then decanted and recentrifuged in an ultracentrifuge[11] at 105,000 X g for 90 minutes at 4 C. The pink pellet sediment (microsomes) was collected from the bottom of the tubes and resuspended in Tyrode solution (pH 7.4). In one experiment (Table 12) the spleen fragments were homogenized without prior freezing and thawing.

Extraction of Total RNA from the Spleen

The hot phenol procedure used to extract spleen total RNA was a slight modification of the method of Lang and Sekeris (1964). Thirty spleens were each cut into 3 pieces and placed in a 10 ml test tube with 1 ml of Tyrode solution. The spleen fragments were quickly frozen by placing the tube in a dry ice-95% ethanol mixture. The tissue was then

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[10]Ivan Sorvall, Inc., Norwalk, Connecticut.

[11]Beckman, Model L Preparative Ultracentrifuge.

thawed under tap water and homogenized immediately in a 15 ml glass Potter Elvehjem tissue grinder with 20 ml of 0.03 M phosphate buffer (pH 6.8) containing 0.03% sodium deoxycholate, 0.1% polyvinyl sulfate,[12] and 0.14 M sodium chloride. The mixture was then transferred to a 150 ml Erlenmeyer flask and 20 ml of 88% phenol[13] were added and mixed thoroughly. The flask was then incubated for 15 minutes in a 50 C water bath with shaking. After incubation, 20 ml of 0.9% NaCl solution were added and the mixture was centrifuged at 27,000 X g for 30 minutes in a Servall RC-2 refrigerated centrifuge with rotor SS-34. The upper aqueous layer (RNA I), which contained r-RNA and t-RNA, was removed. The interphase was extracted with 20 ml of buffer and then mixed with 20 ml of 88% phenol at 65 C for 15 minutes in a shaking water bath, as before. The interphase fraction was then combined with 20 ml of 0.9% NaCl solution and centrifuged at 27,000 X g for 15 minutes. The aqueous layer (RNA II) was carefully collected and combined with the RNA I fraction.

The combined aqueous solutions were extracted again with 88% phenol at room temperature for 15 minutes on a shaker. The mixture was then centrifuged at 4,000 X g for 5 minutes. The aqueous upper layer and the interphase (which contained the RNA) were transferred to a flask and shaken five times with equal volumes of peroxide-free ether to remove phenol. Nitrogen was then bubbled through the solution until it was free of ether. The RNA was then precipitated by addition of two volumes of absolute ethanol and was stored at -20 C overnight. The total RNA extract was concentrated by centrifugation and dissolved in 0.9% NaCl for

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[12] K & K Lab., Inc., Plainview, N. Y.

[13] Mallinckrodt Co., St. Louis, Missouri.

injection.

RNA Determination

The isolated RNA fraction was characterized by ultraviolet absorption (Ogur and Rosen, 1950) and by the Orcinol test (Ceriotti, 1955).

A sample was diluted 15 times with 10% perchloric acid solution and scanned with a Beckman DB spectrophotometer in the ultraviolet absorption region from 200 through 320 mμ. An ultraviolet absorption peak at 258 to 260 mμ indicated the presence of nucleic acid.

The assay for pentose in the RNA extract (Orcinol test) was performed as follows: 200 mg of Orcinol[14] (m.p. 106 C) was dissolved in concentrated hydrochloric acid, 10 ml of $CuCl_2 \cdot H_2O$ solution was added, and the volume was made up to 100 ml with concentrated hydrochloric acid. Five ml of this reagent were combined with 5 ml of the solution to be tested. The test tubes were shaken thoroughly and immersed in a boiling water bath for 40 minutes. The tubes were then cooled under running water. The color was extracted with 5 ml of isoamyl alcohol and, after centrifugation, was read with a Beckman DB spectrophotometer at 675 mμ against a blank treated in the same manner. A standard curve was constructed using a commercially obtained RNA standard prepared from Torula yeast.[15] Serial dilutions of the RNA standard were made in the buffered saline to give a range of 2 to 40 μg of RNA per ml. The optical density of the unknown solution was converted to concentration of RNA by reference to the standard curve. The average quantity of RNA per spleen was

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[14]Fisher Scientific Co., Philadelphia, Penn.

[15]Sigma Chemical Co., St. Louis, Missouri.

0.28 mg.

Transfer of Immunity

Three weeks after the second immunizing egg dose, the donor spleen cells or spleen cell fractions were used in the transfer of immunity experiments. Normal, non-immune adult mice were used as recipients. Most of the recipients were irradiated, but some were non-irradiated. Twenty-four hours after irradiation the latter type of recipients were injected intravenously with 1.0 ml of Tyrode solution containing either 4×10^7 bone marrow cells or 1×10^8 spleen cells from normal syngeneic mice. In the experiments on transfer of immunity with spleen cells, spleen cells from immunized donors were given instead. Before being given an intravenous injection, each mouse was heat-treated by exposure to an infrared lamp[16] for 1 minute. It was then confined in a holder with its tail extended outside. Injection was made into the tail vein with a 1 ml tuberculin syringe with a Luer Lock connector fitted with a 27-gauge needle. To test the transfer of immunity with cell fractions, freshly prepared homogenate or cell fractions were injected intraperitoneally, except in one experiment where one group of mice was given the microsome fraction by intravenous injection. Recipient mice were given a second transfer one week later. A challenge dose of about 5,000 H. nana eggs was administered 20 days after the first transfer of cellular material to determine the susceptibility of the recipient to infection.

In the initial experiments, in which the spleen-cell recipients were x-irradiated, the challenge controls were x-irradiated and implanted

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[16]Fisher Infra-Rediator, 500 W. Heat Reflector, Fisher Scient. Co., Philadelphia, Penn.

with normal spleen cells. Since controls so treated were found to be as susceptible to H. nana infection as the normal untreated mice, normal non-irradiated mice were used as challenge controls in subsequent experiments.

Parasite Count

Cysticercoid counts were made essentially as described by Hunninen (1935b). Mice were killed by cervical dislocation 90 to 92 hours after egg challenge. The entire small intestine was removed from each mouse, placed in a petri dish containing tap water, and stored in a refrigerator for two days to allow autolysis to proceed. All fat and adherent blood vessels were then stripped from the intestine with a small, sharp forceps. The intestine was slit open, shaken briskly in a steel pan containing about 3,000 ml of tap water to rid it of mucus, then cut into 5 cm lengths. The flattened sections of intestine, with the tips of the villi up, were placed in petri dishes and covered with just enough tap water to allow the villi to float. The cysticercoids, which were in the villi, were counted under a dissecting microscope.

Statistical Analysis

The Mann-Whitney U Test (one-tailed) was used to analyze the data (Siegel, 1956). Differences between groups were considered significant at the 5% probability level.

CHAPTER III

RESULTS

The purpose of the initial experiments was to compare the effect different routes of administration of immunizing egg doses had on development of resistance to a standard challenge infection. The results of these studies are presented in Tables 1, 2, and 3. Resistance to the challenge infection was complete regardless of whether the immunizing egg doses were given by the oral, the intraperitoneal, or the intravenous routes, or by both the oral and intraperitoneal routes.

Although parts I and II of the experiment shown in Table 3 were performed at different times, it is apparent that the mice given two intravenous egg doses resisted the challenge infection better than those given only one dose.

The next four experiments were designed to investigate various factors which influence adoptive transfer immunity to H. nana with spleen cells. Results presented in Tables 4 and 5 show the effect of the route of immunication of the donors on the level of immunity transferred. Transfer of immunity was successful when the spleen-cell donors were given two immunizing egg doses by either the oral or intravenous routes. Relatively little (Table 4) or no immunity (Table 5) was transferred when the immunizing egg doses were injected intraperitoneally or when an

TABLE 1

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN MICE IMMUNIZED BY DIFFERENT ROUTES[a]

Challenge controls (1)	Route of immunization[b]		
	Oral (2)	IP (3)	Oral + IP (4)
179	0	0	0
196	0	0	0
225	0	0	0
251	0	0	0
258	0	0	0
262	0	0	0
301	0	0	0
311	0	0	0
328	0	0	0
375	0	0	0

[a]Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge with 4,900 H. nana eggs. Challenge given 3 weeks after the second immunizing egg dose.

[b]Immunizing egg doses: (2) 4,850 oral, 3 weeks later 9,400 oral; (3) 4,850 IP, 3 weeks later 9,400 IP; (4) 4,850 oral and 4,850 IP, 3 weeks later 9,400 oral and 9,400 IP.

TABLE 2

CYSTICERCOID COUNTS AFTER EGG CHALLENGE AS AFFECTED BY THE SEQUENCE OF IMMUNIZATION VIA THE ORAL AND INTRAPERITONEAL ROUTES[a]

Challenge controls (1)	Route and sequence of immunization[b]			
	2 oral (2)	2 IP (3)	2 oral + 2 IP (4)	2 IP + 2 oral (5)
189	0	0	0	0
212	0	0	0	0
243	0	0	0	0
304	0	0	0	0
318	0	0	0	0
359	0	0	0	0
376	0	0	0	0
409	0	0	0	0
458	0	0	0	0
473	0	0	0	0

[a]Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge with 5,180 H. nana eggs. Time of challenge after the last immunizing egg dose: (2) and (3) 9 weeks; (4) and (5) 3 weeks.

[b]Immunizing doses of eggs given at 3 week intervals: (2) 5,210 oral, 9,800 oral; (3) 5,210 IP, 9,800 IP; (4) 5,210 oral, 9,800 oral, 6,150 IP, 12,400 IP; (5) 5,210 IP, 9,800 IP, 6,150 oral, 12,400 oral.

TABLE 3

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN MICE IMMUNIZED VIA THE INTRAVENOUS ROUTE[a]

Part I[b]		Part II[c]	
Challenge controls (1)	Immunized mice (2)	Challenge controls (3)	Immunized mice (4)
334	7	91	0
369	33	95	0
d 380	38	102	0
456	102	132	0
493	121	135	0
575	216	159	0
		182	0
		185	0
		201	0
		241	0

[a]Each entry is the number of cysticercoids in a single mouse 90-92 hours after egg challenge.

[b]Test mice given one immunizing egg dose of 9,000 eggs by IV injection and challenged 3 weeks later with 5,400 eggs.

[c]Test mice given two immunizing egg doses by IV injections: 4,500 initially and 3 weeks later 9,400. Mice challenged with 4,000 eggs 3 weeks after the second immunizing egg dose.

[d]Median

TABLE 4

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN X-IRRADIATED MICE IMPLANTED WITH SPLEEN CELLS FROM DONORS IMMUNIZED BY DIFFERENT ROUTES[a] (EXPERIMENT I)

Treatment of donors[b]			
None (1)	Oral Eggs (2)	IP eggs (3)	Oral + IP eggs (4)
281	0	148	151
295	0	179	204
329	6	215	237
340[c]	15	233	243
422	18	256	285
579	18	262	319
689	36	290	662
809	153	294	-[d]

[a]Spleen cells ($1X10^8$) transplanted 3 weeks after donors given the last immunizing egg dose. Recipients irradiated (950R) 1 day before cells implanted, and challenged with 5,000 H. nana eggs 20 days after cells implanted. Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge. Differences between groups at 5% probability level: (1) versus (2), (3) or (4) significant; (2) versus (3) or (4) significant.

[b]Egg doses given to spleen cell donors: (1) none; (2) 5,000 oral, 3 weeks later 9,950 oral; (3) 5,000 IP, 3 weeks later 9,950 IP; (4) 5,000 oral and 5,000 IP, 3 weeks later 9,950 oral and 9,950 IP.

[c]Median

[d]Mouse died before the end of the experiment.

TABLE 5

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN X-IRRADIATED MICE IMPLANTED WITH SPLEEN CELLS FROM DONORS IMMUNIZED BY DIFFERENT ROUTES[a] (EXPERIMENT II)

Treatment of donors[b]				
None (1)	Oral eggs (2)	IP eggs (3)	Oral + IP eggs (4)	IV eggs (5)
91	5	38	60	49
95	20	86	71	55
102	21	90	86	58
132	24	106	93	60
135 [c]	35 →	118 →	107 →	→ 67
159	42	132	113	75
182	47	134	119	78
185	50	141	143	104
201	63	145	157	110
241	72	146	220	-[d]

[a]Spleen cells ($1X10^8$) transplanted 3 weeks after donors given last immunizing egg dose. Recipients irradiated (950R) 1 day before cells implanted, and challenged with 4,800 H. nana eggs 20 days after cells implanted. Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge. Differences between groups at 5% probability level: (1) versus (2) or (5) and (2) versus (3), (4) or (5) significant; (1) versus (3) or (4) not significant.

[b]Egg doses given to spleen cell donors: (1) none; (2) 6,000 oral, 3 weeks later 11,500 oral; (3) 6,000 IP, 3 weeks later, 11,500 IP; (4) 6,000 oral and 6,000 IP, 3 weeks later 11,500 oral and 11,500 IP; (5) 6,000 IV, 3 weeks later, 11,500 IV.

[c]Median

[d]Mouse died before the end of the experiment.

intraperitoneal injection of eggs was given immediately after each of two oral egg doses.

Results presented in Table 6 show cysticercoid counts after egg challenge in two groups of non-immunized mice, one of which was irradiated (950R) and given normal spleen cells one day later. There was no significant difference between the two groups in infection rates. Thus, either 950R x-radiation had no effect on the natural immunity or, if there was some loss of natural immunity, it was regained by the time of the egg challenge 20 days after spleen cell implantation.

The effect of pre-irradiation of the cell recipient on transfer of immunity with spleen cells is shown in Table 7. Fewer cysticercoids developed after challenge in the irradiated recipients than in non-irradiated mice. Thus it appears that pre-irradiation of cell recipients promotes transfer of immunity to H. nana.

The next experiment was carried out to determine whether antigenic material in sufficient quantity to actively immunize non-irradiated recipients is transferred with the usual number of spleen cells used in the adoptive immunity experiments. Since the immunized spleen cell donors were C_3BF_1 mice and the cell recipients were CD_1 mice, it is to be expected that the donor cells would be rejected by a homograft reaction (Boyd, 1966; Smith and Congdon, 1960), and adoptive transfer of immunity would be precluded. The results (Table 8, groups 3 and 4) show that the non-immunized CD_1 mice did not become immunized after injection with spleen cells from immunized C_3BF_1 donors. This indicates that if there was any antigen carried over in the cell transfer, the amount was not sufficient to immunize the CD_1 recipients.

TABLE 6

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN NORMAL NON-IRRADIATED CONTROLS AND IN X-IRRADIATED MICE IMPLANTED WITH NORMAL SPLEEN CELLS[a]

Non-irradiated	X-irradiated + spleen cells[b]
55	91
115	95
122	102
139	132
139	132
141 [c] →	135 →
164	159
169	182
181	185
187	201
205	241

[a]Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge with 4,800 H. nana eggs. Difference between groups at 5% probability level was not significant.

[b]Mice irradiated (950R) and implanted with syngeneic spleen cells ($1X10^8$) from non-immunized donors 1 day later. Egg challenge given 20 days after cell implantation.

[c]Median

TABLE 7

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN X-IRRADIATED AND NON-IRRADIATED MICE IMPLANTED WITH SPLEEN CELLS FROM IMMUNIZED DONORS[a]

Challenge controls (1)	Treatment of cell recipients[b]	
	X-irradiated (2)	Non-irradiated (3)
135	68	103
146	71	148
147	84	160
155	104	183
→ 212[c]	→ 108	→ 185
221	119	188
232	127	192
289	152	216
318	160	251

[a]Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge with 5,250 H. nana eggs. Differences between groups at 5% probability level: (1) versus (2) and (2) versus (3) significant; (3) versus (1) not significant.

[b]Recipients received spleen cells ($1X10^8$) from donor mice which had been given two oral immunizing egg doses: 5,000 and 3 weeks later 12,000. (2) irradiated (950R) 1 day before cell implantation.

[c]Median

TABLE 8

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN CD_1 MICE IMPLANTED WITH SPLEEN CELLS FROM IMMUNIZED C_3BF_1 DONORS[a]

Challenge controls		Type of spleen cells implanted	
Non-immunized (1)	Immunized[b] (2)	Non-immunized[c] (3)	Immunized[d] (4)
73	0	90	85
90	0	103	111
102	0	121	113
105	0	→ 124	115
[e] → 129	0	150	→ 132
134	0	167	143
139	0	199	158
156	0	-[f]	171
172	0	-	190
212	1	-	-

[a]Each entry is the number of cysticercoids in a single CD_1 mouse 90-92 hours after challenge with 4,950 H. nana eggs. All four groups challenged at the same time, 3 weeks after spleen cell implantation from C_3BF_1 donors.

[b]Immunized challenge controls given 5,000 eggs orally 3 weeks before egg challenge.

[c]Non-immunized CD_1 recipients implanted with $1X10^8$ spleen cells from non-immunized C_3BF_1 donors.

[d]Non-immunized CD_1 recipients implanted with $1X10^8$ spleen cells from immunized C_3BF_1 donors. The C_3BF_1 cell donors immunized by oral administration of 5,100 eggs and 2 weeks later 9,500 eggs.

[e]Median

[f]Mouse died before the end of the experiment.

The results of previous experiments (Tables 4 and 5) suggested that intraperitoneal administration of eggs made the spleen cells immunologically unresponsive to H. nana. The purpose of the next experiment (Table 9) was to learn if this immunologic unresponsiveness was maintained when the spleen cells from donors injected with eggs intraperitoneally were transplanted into irradiated recipients. Mice were given two intraperitoneal injections of H. nana eggs and three weeks after the second injection their spleen cells were transplanted to non-immunized irradiated recipients. The spleen cell recipients, which were given an immunizing oral egg dose one day after cell implantation, completely resisted the egg challenge given 19 days after immunization. The results indicate that induced unresponsiveness to H. nana was broken either in the donors before the cells were transplanted or in the recipients within 24 hours after transplantation.

The purpose of the next experiment was to investigate the persistance of pre-irradiation acquired immunity to H. nana in lethally irradiated mice restored with spleen cells from donors given two intraperitoneal injections of eggs and in similarly immunized and irradiated recipients given normal spleen cells. The results are shown in Table 10. Both groups of animals were completely resistant to the challenge infection given 20 days after the cell implantation.

The last phase of the research was designed to investigate the possibility of transfer of immunity to H. nana with spleen cell fractions from immunized donors. Table 11 shows results obtained with mice given normal spleen cells one day after 950R x-irradiation and the next day injected with either spleen homogenate or microsome fraction from immu-

TABLE 9

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN X-IRRADIATED MICE IMPLANTED WITH SPLEEN CELLS FROM INTRAPERITONEALLY IMMUNIZED DONORS AND ONE DAY LATER GIVEN AN ORAL EGG DOSE[a]

Challenge controls (1)	Treatment of spleen cell donors	
	IP eggs[b] (2)	None (3)
80	0	0
90	0	0
109	0	0
114	0	0
158	0	0
176	0	0
217	0	0
247	0	0
248	0	0
366	0	-[c]

[a]Spleen cells ($1X10^8$) transplanted 3 weeks after donors given second immunizing egg dose. Recipients irradiated (950R) 1 day before cells implanted and given an immunizing oral egg dose of 5,000 one day after cell implantation. Egg challenge given 20 days after cells implanted. Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge with 5,260 H. nana eggs.

[b]Donors given two oral immunizing egg doses: 5,100 initially and 3 weeks later 9,500.

[c]Mouse died after spleen cell injection.

TABLE 10

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN PREIMMUNIZED-IRRADIATED MICE IMPLANTED WITH SPLEEN CELLS FROM INTRAPERITONEALLY IMMUNIZED DONORS OR FROM NON-IMMUNIZED DONORS[a]

Challenge controls (1)	Treatment of spleen cell donors	
	IP eggs[b] (2)	None (3)
145	0	0
149	0	0
172	0	0
224	0	0
239	0	0
255	0	0
285	0	0
296	0	0
307	0	0
358	-[c]	0

[a]Recipients immunized with 5,000 H. nana eggs orally 3 weeks before irradiation (950R). Spleen cells ($1X10^8$) transplanted 1 day after irradiation. Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge with 5,260 eggs 3 weeks after irradiation.

[b]Donors given two IP immunizing egg doses: 5,300 initially and 3 weeks later 5,000.

[c]Mouse died after spleen cell injection.

TABLE 11

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN X-IRRADIATED MICE INJECTED WITH NORMAL SPLEEN CELLS AND THEN WITH SPLEEN CELL FRACTIONS FROM IMMUNIZED DONORS[a]

Challenge controls (1)	950R + $1X10^8$ whole cells from immunized donors (2)	950R + normal spleen cells + cell fraction from immunized donors[b]		
		Homogenate (IP) 1 spleen equivalent (3)	Microsome (IP) 2 spleen equivalent (4)	Microsome (IP) 2 spleen equivalent (5)
119	64	11	69	0
135	77	34	87	98
140	90	35	→ 128	→ 135
154	95	35	140	142
160	101	→ 42	153	166
→ [c] 167	→ 115	108	-[d]	-
180	126	144	-	-
226	149	160		-
263	158	182		-
274	187	-		

[a]Spleen cells ($1X10^8$) transplanted 3 weeks after donor given last immunizing egg dose. Recipient irradiated (950R) 1 day before $1X10^8$ normal spleen cells implanted and challenged with 5,660 H. nana eggs 20 days after cells implanted. Differences between groups at 5% probability level: (1) versus (2), (3) or (4) significant; (1) versus (5) not significant.

[b]Donors given two oral immunizing egg doses: 5,230 initially and 3 weeks later 11,800.

[c]Median

[d]Mouse died 2 days after cell fraction injected.

nized donors. Cysticercoid counts after egg challenge indicated that a significant level of immunity was induced in the non-immunized recipients by both the homogenate (group 3) and the intraperitoneally administered microsome fraction (group 4). The median cysticercoid count in the mice that received the intravenously injected microsomes (group 5) was lower than that in the challenge controls but the difference in number of parasites between the two groups was not significant at the 0.05 probability level. For unknown reasons, the microsomal injection killed some of the recipients.

The results of cysticercoid counts in mice treated the same way with respect to irradiation and post-irradiation spleen cell implantation as those in the previous experiment are presented in Table 12. The animals were further given two injections of microsomes from immunized or from non-immunized donors. The quantity of microsomes in each of the two injections was less than the amount given in a single injection in the previous experiment (Table 11). This was done in order to avoid the lethal side effect noted previously. Due to unavailability of normal mouse serum, serum from previous infected mice was used in the process of homogenization of spleen cells in the second microsome transfer. The same serum was used to prepare microsomes from immunized and non-immunized donors. It is felt that an insignificant amount of the serum was present in the microsome recipients at the time of egg challenge because: (1) a total of 0.5 ml of serum was used to prepare microsomes for 10 mice, (2) the microsomes were isolated by centrifugation and the supernatant was discarded, (3) more than 99.9% of any serum that was injected would be lost in 20 days by biological breakdown (Dixon et al., 1952b), and (4)

TABLE 12

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN X-IRRADIATED MICE INJECTED WITH NORMAL SPLEEN CELLS AND THEN WITH SPLEEN MICROSOMES FROM SENSITIZED OR NORMAL DONORS[a]

	950R + normal spleen cells + microsomes	
Challenge controls (1)	Sensitized microsomes[b] (2)	Non-sensitized microsomes[c] (3)
57	63	97
123	92	134
135	95	140
155	109	160
158 [d] →	113 →	169 →
178	115	172
187	133	182
199	134	184
227	136	189
271	149	289

[a]Spleen cells ($1X10^8$) transplanted 3 weeks after donors given last immunizing egg dose. Recipients irradiated (950R) 1 day before cells implanted and challenged with 5,000 H. nana eggs 20 days after cells implanted. Differences between groups at 5% probability level: (1) versus (2) and (2) versus (3) significant; (1) versus (3) not significant.

[b]Donors given two oral immunizing egg doses: $5X10^3$ and $1X10^4$. Recipients injected IP with 1 spleen equivalent of microsomes 1 day after spleen cells implanted and 1 week later with 1.5 spleen equivalents of microsomes.

[c]Same quantity of microsomes and injection schedule as group (2) but donors were non-immunized.

[d]Median

from 2.6 ml to 4 ml of immune serum is required to passively immunize a mouse to H. nana (Hearin, 1941). The results indicate that a significant level of immunity was induced in the non-immunized x-irradiated recipients by injection of microsomes from the immunized donors (group 2) but no significant immunity was induced by the normal microsomes (group 3).

Induction of immunity by injecting microsomes from immunized or non-immunized donors was studied in irradiated mice treated with normal spleen cells and in non-irradiated mice (Table 13). Immunity was induced in the mice given sensitized microsomes, as indicated by comparison with challenge controls, regardless of whether the recipients were irradiated or non-irradiated (groups 2 and 4). No significant immunity was induced in mice given non-sensitized microsomes (groups 3 and 5).

An attempt was made to transfer immunity to irradiated, marrow-treated mice with spleen homogenate or RNA. The recipients were exposed to 950R x-radiation and the next day they were injected with normal syngenic marrow. They were further injected with either spleen homogenate or RNA from immunized donors. The results are shown in Table 14. Based on comparison with challenge controls (group 1), no significant immunity was transferred in either of these two groups (groups 3 and 4). As in previous experiments, good immunity was transferred to the x-irradiated recipients implanted with spleen cells from immunized donors (group 2). Five of nine mice in group 2 died shortly after the injection of the spleen cells. The reason is unknown, but embolism is thought to be the cause. This is known to occur if an excessive number of injured cells are injected intravenously.

TABLE 13

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN NORMAL MICE AND IN IRRADIATED MICE IMPLANTED WITH NORMAL SPLEEN CELLS AS AFFECTED BY INJECTION OF SENSITIZED OR NON-SENSITIZED MICROSOMES[a]

	950R + $1X10^8$ spleen cells + microsomes		Microsomes only	
Challenge controls (1)	Sensitized microsomes (2)	Non-sensitized microsomes (3)	Sensitized (4)	Non-sensitized (5)
85	77	62	59	52
94	83	82	71	100
118	112	87	84	111
155	114	116	92	136
182[b]	→ 116	→ 130	104 →	142 →
237	119	133	136	145
241	136	141	147	156
244	142	248	156	164
260	154	257	158	190
270	-[c]	-	230	193

[a]Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenge with 5,100 H. nana eggs. Challenge given 2 weeks after the second microsome injection. Irradiated recipients (groups 2 and 3) exposed to 950R and 1 day later implanted with $1X10^8$ non-sensitized spleen cells. Microsome donors for groups 2 and 4 given 2 oral immunizing egg doses: $5X10^3$ and 3 weeks later $1X10^4$. All microsome recipients injected IP with 2.25 spleen equivalents of microsomes 1 day after spleen cells implanted and 1 week later with 3 spleen equivalents of microsomes. Differences between groups at 5% probability level: (1) versus (2) or (4) significant; (1) versus (3) or (5) not significant; (2) versus (3) and (4) versus (5) not significant.

[b]Median

[c]Mouse died before the end of the experiment.

TABLE 14

CYSTICERCOID COUNTS AFTER EGG CHALLENGE IN X-IRRADIATED MICE INJECTED WITH MARROW CELLS AND WITH SPLEEN HOMOGENATE OR RNA FROM IMMUNIZED DONORS[a]

		950R + marrow + spleen cell fraction from immune donors[b]	
Challenge controls (1)	950R + sensitized spleen cells[b] (2)	Homogenate (1 spleen equivalent) (3)	RNA (2 spleen equivalents) (4)
95	39	88	83
101	63	120	143
111	82	141	145
196	89	142	156
→ 208	-[d]	→ 144	161
228	-	185	187
239	-	193	215
250	-	223	246
294	-	341	-

[a]Cell recipients irradiated (950R) and 1 day later implanted with $1X10^8$ spleen cells (group 2) or $4X10^7$ marrow cells (groups 3 and 4). Mice in groups (3) and (4) were further injected with spleen cell homogenate or RNA from immunized donors 1 day after cells implanted. All mice were challenged 19 days after injection of the cell fraction. Each entry is the number of cysticercoids in a single mouse 90-92 hours after challenged with 5,034 H. nana eggs. Differences between groups at 5% probability level: (1) versus (2) significant; (1) versus (3) or (4) not significant.

[b]Spleen donors were immunized by two oral egg doses: 6,000 initially and 3 weeks later 13,600.

[c]Median

[d]Mouse died before the end of the experiment.

CHAPTER IV

DISCUSSION

The results of the experiment on the effect of the route of egg administration on immunization (Tables 1, 2 and 3) indicate that the oral, intraperitoneal, intravenous, and combinations of the oral and intraperitoneal routes are all effective in inducing resistance to oral egg challenge. In contrast, distinct differences were observed between the intraperitoneal route and either the oral or intravenous routes when adoptive transfer of immunity with spleen cells and oral egg challenge of the cell recipients was the test system (Tables 4 and 5). An effective level of adoptive immunity was noted when the spleen cell donors were immunized by two oral egg doses or by two intravenous egg doses, but relatively little or no immunity could be transferred when an intraperitoneal egg dose immediately followed each of the two egg doses or when the donors were given two egg doses by intraperitoneal injection alone.

These results suggest that the administration of H. nana eggs by the intraperitoneal route induced selective tissue immunologic unresponsiveness and responsiveness in the same mice, i.e., the immunologically competent cell population of the spleen was made unresponsive to H. nana antigen, whereas the competent cells of other lymphoid tissue (probably in the region of the small intestine) were sensitized.

According to a current concept (Dresser and Mitchison, 1968; Miller, 1968), for immunization to occur, the antigen must be processed by macrophage or dendritic reticulum cells before it encounters immunocompetent cells. Immunologic unresponsiveness (tolerance) is induced if the antigen reaches the immunocompetent cells before it is processed. It is not known whether tolerance results from cell lysis and deletion of the clone of cells capable of responding to a particular antigen or by a modification of these cells so that they can not be sensitized.

An explanation of the dual response observed in the present study, consistent with the concept described above, is that sufficient antigen (from the larval stage of the parasite) derived from eggs injected intraperitoneally had direct access to immunocompetent cells of the spleen to reduce their number below the minimum required for adoptive transfer of immunity and that comparatively little, or no antigen derived from intraperitoneally injected eggs had direct access to the immunocompetent cells responsible for resistance to oral egg challenge. Presumably, when eggs are administered by the oral route, sufficient processed antigen reacts with immunocompetent spleen cells to make possible a good adoptive transfer of immunity.

A higher level of adoptive immunity to H. nana was transferred to x-irradiated spleen cell recipients than to non-irradiated cell recipients (Table 7). A similar observation was made by Mäkela and Mitchison (1965) in their study on adoptive transfer of immunity to BSA in syngeneic mice. They noted that pre-irradiation of the recipients of primed lymph-node cells or spleen cells resulted in about a 10-fold increase in antibody titer. Celada (1966) showed that the "barrier" to adoptive transfer

of immunity which is observed in mature syngeneic mice does not exist in newborn mice but develops gradually as the animal matures. Irradiation abolished this "barrier." Other workers have also reported that the use of irradiated recipients increases the expression of immunologic potential in adoptive transfer experiments (Makinodan et al., 1956; Roberts et al., 1957). The nature of the "barrier" is not understood. The reason why irradiation enhances adoptive transfer of immunity is also not clear, but it is possible that: (1) there is more space for cell repopulation in the radiation-depleted tissue, or (2) cells injured by radiation release factors that stimulate function and/or multiplication of the transplanted cells (Harris et al., 1954; Johnson et al., 1968).

The results of the experiment in which pre-irradiation acquired immunity to H. nana persisted after lethal irradiation (950R) in mice restored with normal spleen cells (Table 10) extends the work of Friedberg et al. (1970). The latter authors showed that 950R x-irradiation depressed acquired immunity to H. nana and that transplanted syngeneic marrow from donors mitigated this effect. The mechanism is not understood but it is possible that: (1) injected hemopoietic cells provide immunologically competent cells either directly or indirectly via stem cells and persisting antigen or an immunologically committed cell fraction (e.g., antigen-RNA complex or information RNA) derived from the irradiated host cells reacts with the transplanted immunocompetent cells, or (2) the transplanted cells (from non-immunized donors) repopulate the radiation-depleted tissues and restore the normal cell interaction required for an immune response (Friedberg et al., 1970; Bosma et al., 1968).

Pre-irradiated recipients of spleen cells from donors given H. nana eggs intraperitoneally were immunologically competent when tested 24 hours after the cells were implanted (Table 9). The transplanted spleen cells are considered responsible for the immune capacity because mice cannot be immunized to H. nana two days after 950R, the radiation dose used in this experiment (personal communication from W. Friedberg). Thus, the results of this experiment indicate that the induced unresponsiveness to H. nana in spleen cells in mice given eggs intraperitoneally, as described previously (Tables 4 and 5), was terminated either before the cells were transplanted or within 24 hours after transplantation. Presumably this occurred when new immunocompetent cells were propagated from stem cells.

It was shown (Tables 4 and 5) that spleen cells from mice given intraperitoneal egg doses could transfer only a very low level, if any, of immunity to irradiated recipients. From this observation, and the observation (Table 9) that induced unresponsiveness is broken in spleen cells by 24 hours after transplantation, one would expect that spleen cells from donors given eggs intraperitoneally would function in the same way as normal spleen cells in promoting persistence of pre-irradiation immunity. This is in agreement with the results (Table 10). Thus, the immunity observed in the pre-immunized irradiated mice implanted with spleen cells from donors given eggs intraperitoneally was mainly, if not entirely, due to host cells, or was derived from host cells.

Mice injected with spleen-cell homogenate or the microsome fraction from immunized donors resisted H. nana infection better than untreated mice (challenge controls), whereas infection rates in animals given

microsomes from non-immunized donors were not significantly different from those in the challenge controls (Tables 11, 12, and 13). Although these results suggest that immunity to H. nana can be transferred with spleen-cell fractions, the possibility of non-specific enhancement of resistance to H. nana has not been excluded.

In two experiments, microsomes from non-immunized and immunized donors were compared. The results of the first study (Table 12) indicated that the sensitized microsomes were more effective than non-sensitized microsomes in inducing resistance to H. nana. In the second experiment (Table 13), the difference between non-sensitized and sensitized microsomes was not significant, although the animals that received the sensitized microsomes had significantly lower infection rates than the challenge controls. More decisive results might be obtained in a future study by using larger numbers of mice and by administration of equal amounts of cell-fractions as measured by protein nitrogen and/or RNA rather than spleen equivalents, as in the present study.

The question of whether the induced resistance to H. nana in the mice that received cell fractions was due to the presence of viable sensitized cells was resolved in a separate experiment (Table 14). Lethally irradiated mice restored with implanted syngeneic bone marrow were injected with spleen homogenate from immunized donors. The significance of this test is that the x-irradiation suppressed the immune capacity and the implanted marrow saved the mice from hematopoietic death (DeVries and Vos, 1959; Smith and Congdon, 1960) but did not provide the cellular elements necessary for an immune response (Claman et al., 1966a; 1966b; Claman et al., 1968). Immunity would be transferred to these mice by

injected spleen homogenate only if viable, sensitized cells were present in the homogenate in sufficient numbers. The homogenate was prepared as described (see Materials and Methods). The results indicate that immunity was not transferred to the irradiated, marrow-treated mice with the spleen homogenate. With intact spleen cells from the same group of donors, transfer of immunity was achieved. It is concluded, therefore, that few, if any, viable sensitized cells were present in the homogenate. For the microsomes, the isolation procedure itself, which involved differential centrifugation, precludes the presence of whole cells. By inference, intact cells are not responsible for the resistance to H. nana induced by the injection of spleen-cell homogenate or microsomes from immunized donors.

The mechanism by which resistance to H. nana was induced in mice injected with cell-free fractions from immunized donors is not understood, but several possibilities are suggested by studies on other systems. Results of experiments by Askonas and Rhodes (1965) on transfer of immunity to I^{131} labeled hemocyanin and by Friedman (1965) on transfer of immunity to Shigella indicated that transfer of immunity with cell fractions involved antigen or antigen determinants complexed to RNA. Johnson et al. (1968) reported that syngeneic and heterologous nucleic acid, or their breakdown products, were non-specific stimulators of antibody synthesis. A specific, informational role for RNA in the transfer of immunity was described by Adler et al. (1966) and Bell and Dray (1969). Spleen cells from non-immunized rabbits were induced to make antibody by incubating them with RNA from immunized donors, and the antibody synthesized possessed allotypic specificity characteristic of the RNA donor. Some sug-

gested roles for RNA in the transfer of immunity require that it be incorporated into immunocytes. Studies with radioisotope labeled RNA indicate that RNA can be incorporated into cells both *in vitro* and *in vivo* (Amos, 1961; Niu *et al.*, 1961; Schwarz and Rieke, 1962).

There are several possible reasons for the failure of transfer of immunity with the RNA fraction (Table 14): (1) the immune capacity of the irradiated marrow-treated recipients was inadequate (Gregory and Lajtha, 1970), (2) the RNA fraction injected did not carry immunologic information for immunity to *H. nana*, or (3) the RNA fraction was inactivated during preparation.

CHAPTER V

SUMMARY

Transfer of immunity to Hymenolepis nana was investigated in laboratory mice. Donor mice were immunized by various routes. Spleen cells or spleen cell fractions from immunized donors were injected into normal syngeneic recipients which were pre-irradiated (950R) or untreated. All mice were challenged with approximately 5,000 H. nana eggs orally 20 days after injection of the cells or the cell fractions. The degree of immunity induced in the recipient animals was determined by comparison of infection rates in these mice with those in controls.

Mice could be immunized to H. nana by administration of eggs via the intraperitoneal, intravenous, or oral route, or by several combinations of oral and intraperitoneal egg doses. The best adoptive transfer of immunity occurred when spleen cell donors were immunized by the oral route, whereas little or no immunity could be transferred when the donors were given the "immunizing" egg doses by the intraperitoneal route only, or when an intraperitoneal egg dose immediately followed each of two oral egg doses. These results suggest that the administration of H. nana eggs by the intraperitoneal route induced selective tissue immunologic unresponsiveness and responsiveness in the same mice, i.e., the immunologically competent cell population of the spleen was made unresponsive to

H. nana antigen, whereas the competent cells of other lymphoid tissue (probably in the region of the small intestine) were sensitized.

The effect of pre-irradiation of the recipients on adoptive transfer of immunity was studied. A higher level of adoptive immunity to H. nana was transferred to x-irradiated spleen-cell recipients than to non-irradiated cell recipients.

In mice challenged three weeks after 950R irradiation and spleen cell implantation, the susceptibility to H. nana infection was the same as in normal mice. Thus, either 950R x-radiation had no effect on the natural immunity or if there was some loss of natural immunity it was regained by the time of the egg challenge 20 days after spleen cell implantation.

Normal spleen cells and spleen cells from donors given intraperitoneally injected eggs were both effective in the restoration of immune capacity in otherwise lethally irradiated recipients, and in promoting persistence of pre-irradiation-acquired immunity.

The results of an experiment in which spleen cells were transferred from immunized donors to irradiated, allogeneic recipients indicate that, if immunizing antigen was transferred with the spleen cells in the adoptive immunity experiments, the amount was insufficient to actively immunize non-irradiated recipients.

Resistence to H. nana was induced in non-immunized mice by the injection of spleen-cell fractions from immunized donors. Unlike adoptive transfer of immunity with intact spleen cells, irradiation of the recipients and injection of normal spleen cells did not enhance the induced immunity.

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