ELEVATED γ-GLOBULIN AND INCREASED ANTIBODY PRODUCTION IN MICE INFECTED WITH LACTIC DEHYDROGENASE VIRUS

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Plate 50

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Infection of mice with lactic dehydrogenase virus (LDV) resulted in a lifelong elevation of a number of plasma enzymes including lactic dehydrogenase, isocitric dehydrogenase, malic dehydrogenase, phosphohexose isomerase, and glutamic-oxaloacetic transaminase (1). Recent studies on the mechanism of enzyme elevation showed that infected mice cleared endogenous enzymes at a slower rate than uninfected mice (1). The present study was undertaken to see whether the catabolism of a "nonenzymatic" protein, γ-globulin, was also altered. The experiments reported herein show that the catabolism of intravenously administered I^{131} γ-globulin was not impaired in virus-infected mice, but that the level of endogenous γ-globulin was elevated, and that the capacity of the infected mouse to produce antibody to a heterologous protein (human γ-globulin) was enhanced.

Materials and Methods

Animals.—Except where indicated otherwise, conventional CAF-1 male mice, 4 to 6 wk old, obtained from the animal production section of the National Institutes of Health (NIH) were used throughout these experiments. Germfree male and female mice, 3 to 4 wk old, of the NIH stock colony were supplied by Dr. C. E. Miller of the Germfree Unit, Laboratory Aids Branch, and were housed in plastic isolators (2) in the Gnotobiotic Section of the National Institute of Dental Research. All materials introduced into the plastic isolators were treated so as to eliminate viable bacteria. To check for contamination, urine and feces were cultured twice a week.

Virus.—The method of preparation and the assay for LDV have been described previously (3). Stock pools P-16 and P-17 were used throughout these experiments. The titer of the pools was approximately $10^{10.0}ID_{50}$/ml and all dilutions were made in Eagle's basal medium or 0.85% sodium chloride (saline). Mice were infected routinely with LDV by injecting intra-

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peritoneally 0.1 ml of a 10^{-2} dilution of the stock virus. Prior to injection into germfree mice all preparations were passed through a 450 mμ Millipore filter.

Inactivation of Virus.—LDV was inactivated by treating a 10^{-2} dilution of the stock virus with ether, heat, or ultraviolet light. Ether inactivation was performed as described earlier (3, 4). Heat inactivation was carried out by incubating the virus at 80°C for 30 min. Ultraviolet inactivation was performed with a 15 watt GE germicidal lamp placed 10 cm from an open 60 mm Petri dish containing 2.0 ml of the virus. The virus was exposed to the ultraviolet light for 2 min while undergoing constant agitation on a mechanical shaker. Following treatment all preparations were shown to be noninfectious.

Immunoelectrophoresis.—Immunoelectrophoresis of mouse serum was carried out on standard microscope slides coated with 1.0% Ionagar No. 2 (Consolidated Laboratories, Inc., Chicago Heights, Illinois) in Veronal buffer (pH 8.4) under a constant current of 250 v for 90 min. Anti-mouse serum was prepared in rabbits by repeated injections of pooled normal mouse sera in complete Freund's adjuvant (Difco Laboratories, Inc., Detroit, Michigan). Anti-mouse γ-globulin serum (goat) was obtained from Hyland Laboratories, Los Angeles. The slides were photographed 48 hr after addition of the antiserum.

Capillary Precipitins.—The amount of γ-globulin in the serum of infected and uninfected germfree mice was determined by mixing a constant volume of undiluted anti-mouse γ-globulin serum (goat) with serial twofold dilutions of mouse serum in saline. The results are expressed as the reciprocal of the highest dilution of mouse serum giving a visible precipitate in a capillary tube after refrigeration overnight.

Hemagglutination.—The antibody titer in mouse serum following immunization with human γ-globulin was determined by the tanned cell hemagglutination technique of Boyden (5). Three concentrations of crystalline human γ-globulin (4.0, 1.0, 0.25 mg) were used routinely to sensitize 1.0 ml of 2.5% sheep red blood cells. Experiments to be reported separately showed that it was necessary to screen our sera in this way since the concentration of human γ-globulin which gave the highest hemagglutination titer with 7S antibody (γG) was not optimal for obtaining the highest titer with 19S antibody (γM). The data reported in this paper were obtained from experiments in which 4.0 mg of human γ-globulin was employed to sensitize the sheep red blood cells. Hemagglutination titers were read as described by Stavitsky (6). To test for 19S antibody, the mouse serum was incubated at 37°C for 30 min in 0.1 M 2-mercaptoethanol (7) and then tested for loss of hemagglutinating activity.

γ-Globulins.—For the immunization and catabolism studies, human γ-globulin (Cutter, Lot 6708), mouse γ-globulin (Pentex, Lot 461) and rabbit γ-globulin (Pentex) were used. Each preparation gave a single sharp band of precipitation in the 7S γ-globulin region when analyzed by immunoelectrophoresis.

Measurement of Radioactivity.—γ-Globulin was labeled with I^{131} by the iodine monochloride method of McFarlane (8) using approximately 1 mole of I per mole of protein. Mice were injected intravenously or intraperitoneally with 0.5 to 2.0 μc of I^{131}-labeled protein in 0.2 ml saline. To reduce the uptake of I^{131} by the thyroid gland, all mice were given, prior to and throughout the experiment, drinking water which contained 0.01% KI and 0.45% NaCl. Whole body radioactivity was measured by a gamma ray bulk spectrometer (Sharpe Laboratories, La Jolla, California) for 1 min. A radioactive standard of each labeled protein was prepared in saline. The radioactivity of the standard and each animal was measured daily in the whole body counter and the values were corrected for physical decay of the isotope. The per cent of the radioactive protein remaining in the animal on each day was calculated and the results were plotted on semilog paper.

γ-Globulin Catabolism.—The catabolism of γ-globulin in infected and uninfected animals was determined by injecting approximately 50 μg of I^{131}-labeled protein intravenously. The radioactivity in each animal was measured daily and the rate of catabolism was determined

from the graphic plot of the data. Mice were infected with LDV 1 day prior to injection of the labeled protein.

Immune Elimination.—The rate of elimination of a labeled protein from the circulation has been used to measure an immune response in rabbits by Talmage et al. (9) and in mice by Dresser (10). In our experiments each animal was immunized intraperitoneally with 300 μg of γ-globulin. At various times thereafter a tracer dose of I^{131}-labeled protein (approximately 20 μg) was injected intraperitoneally and the per cent of the radioactive protein remaining in each animal was determined daily by whole body counting. Unless specified otherwise, the animals were infected with LDV 1 day prior to immunization.

Histologic Studies.—Germfree mice were infected with LDV and at various times thereafter (1, 3, 6, 10, and 20 days) the cervical, mesenteric, lumbar, axillary, and inguinal lymph nodes and the spleen were removed. Specimens obtained at the same time from uninfected germfree mice served as controls. The specimens were fixed in either neutral buffered formaldehyde or Helly's solution, embedded in paraffin, sectioned at 4 μ, stained with hematoxylin and eosin, and examined with a light microscope.

RESULTS

Elevation of γ-Globulin in Virus-Infected Mice.—The effect of LDV on the level of γ-globulin in the serum of mice was studied by the capillary precipitin method. Germfree mice were employed in these experiments because of their low level of γ-globulin. As seen in Table I, a slight rise in γ-globulin was detected within 6 days after injection of LDV but was more marked at 10 and 20 days. Normal mouse plasma or virus which had been inactivated by heat, ether, or ultraviolet light failed to produce any substantial rise in the level of γ-globulin (Table II). Immunoelectrophoresis of serum obtained 10 days after injection of LDV showed a sizeable precipitin band in the 7S region while

TABLE I

Titer of γ-Globulin in Germfree Mouse Sera after Injection of LDV

Time after injection	Titer* of γ-globulin in mouse sera‡		
	Injected with LDV	Injected with normal mouse plasma	Noninjected
days			
1	<16	ND§	ND
2	<16	ND	ND
3	<16	ND	ND
4	<16	ND	ND
6	32	<16	<16
10	256	<16	<16
20	512	16	<16

* Reciprocal of highest dilution of mouse sera giving a precipitate with goat anti-mouse γ-globulin.

‡ Pooled sera from groups of 4 to 5 mice.

§ ND, not done.

serum from mice which had been injected with normal plasma or inactivated virus failed to show a 7S precipitin band (Text-fig. 1).

Catabolism of γ-Globulin in Infected and Uninfected Mice.—Although antiviral antibody might be responsible for the increase in γ-globulin noted above,

TABLE II

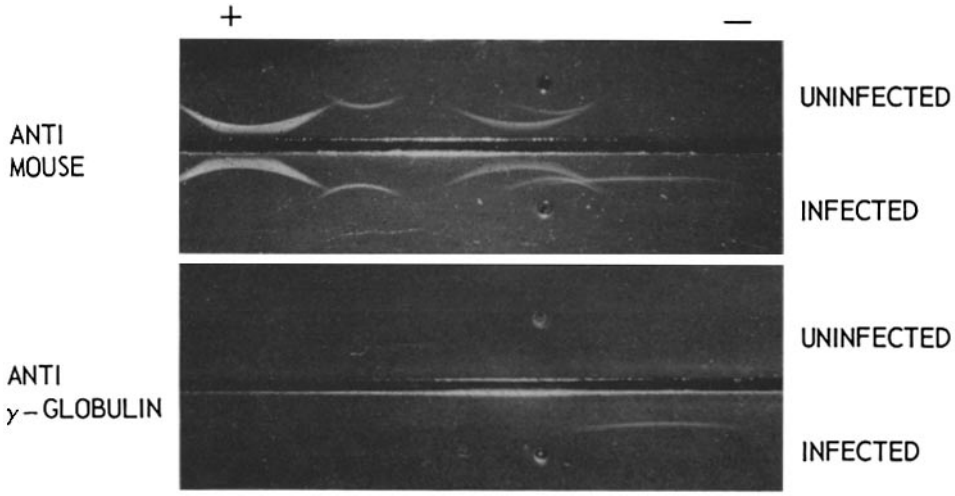
Titer of γ-Globulin in Germfree Mouse Sera 10 Days after Injection of Infectious and Noninfectious Virus

Treatment of LDV prior to injection	Titer* of γ-globulin in mouse sera‡
None	320
Ether§	40
Heat§	40
Ultraviolet light§	80
Normal mouse plasma§	40

* Reciprocal of highest dilution of mouse sera giving a precipitate with goat anti-mouse γ-globulin.

‡ Pooled sera from groups of 4 to 5 mice.

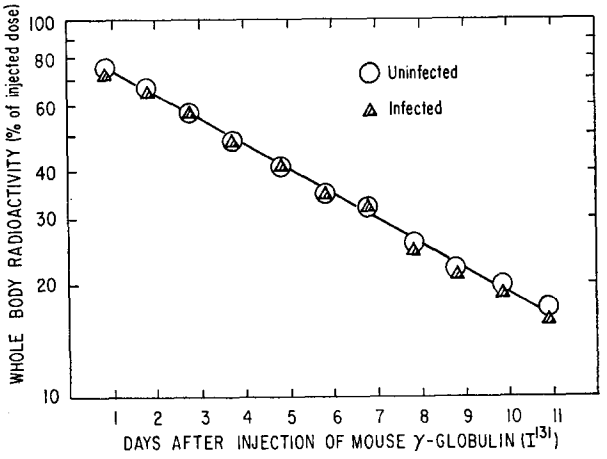
§ Preparations were tested and shown to be noninfectious.



Text-Fig. 1. Immunoelectrophoresis of sera from uninfected and infected (10 days post LDV) germfree mice.

attempts to immunize rabbits and guinea pigs with LDV (1, 11, 12) or to demonstrate neutralizing antibody in mice infected for 1 month (13) have thus far been unsuccessful. Furthermore, virus inactivated by ether, heat, or ultraviolet light failed to raise the level of γ-globulin in germfree mice. These findings together with the recent observation that LDV-infected mice cleared certain proteins (lactic dehydrogenase, etc.) at a slower rate than uninfected mice (1),

suggested that the increase in γ-globulin might be due to impaired clearance of endogenous γ-globulin. To test this possibility, the catabolism of I^{131}–mouse γ-globulin was studied in infected and uninfected mice. As seen in Text-fig. 2, the rate of elimination of the tracer was the same in both groups. These findings argue against impaired clearance as the cause of the elevated γ-globulin. Similarly, the rate of elimination of human γ-globulin was found to be the same in the infected and uninfected mice for the first 4 days, but by day 6 the rate of elimination was markedly accelerated in the infected group (Text-fig. 3). The sudden elimination of the heterologous protein, but not the homologous pro-



Text-Fig. 2. Catabolism of mouse γ-globulin (I^{131}) in infected and uninfected mice. Each group represents the average of 4 mice.

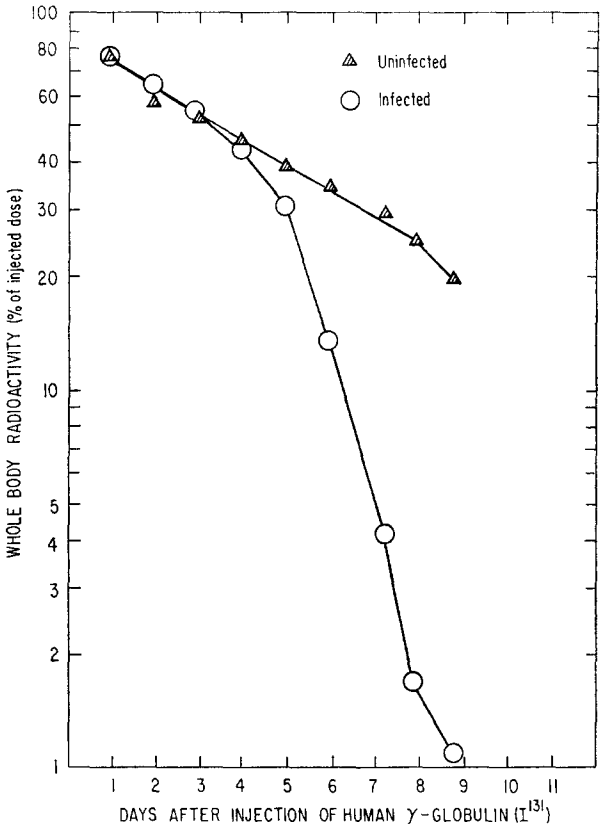
tein, suggested that an immune response had occurred in the virus-infected mice.

Accelerated Immune Response to Heterologous Protein in Virus-Infected Mice.— To study the above observation in more detail, mice were immunized with human γ-globulin. The animals were given a tracer dose of the protein 5, 7, and 9 days later. Although animals immunized with human γ-globulin eliminated the tracer somewhat more rapidly than unimmunized animals, it can be seen from the data in Text-fig. 4 that infection with LDV markedly accelerated the rate of antigen elimination.

To test the immunologic specificity of the response, the rate of elimination of I^{131}–human γ-globulin was compared in groups of mice which had been previously immunized with human or rabbit γ-globulin. As seen in Table III, mice immunized with human γ-globulin catabolized 91% of the I^{131}–human γ-globulin within 24 hr, whereas mice immunized with rabbit γ-globulin catabolized only 27% of the labeled human γ-globulin. Similarly, I^{131}–rabbit

γ-globulin was eliminated more rapidly by mice immunized with the homologous protein.

To substantiate further that the rapid elimination of the antigen in the virus-infected animal was due to antibody, sera was collected at different times after immunization from selected mice used in the immune elimination experiments

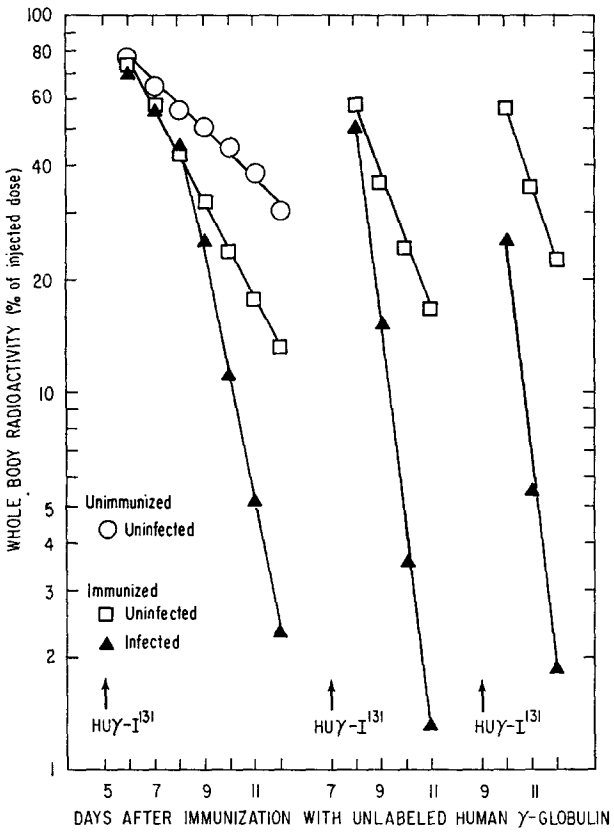


Text-Fig. 3. Catabolism of human γ-globulin (I^{131}) in infected and uninfected mice. Each group represents the average of 4 mice.

(Text-figs. 4 to 6), and was tested for hemagglutinating antibody. The data in Table IV show that antihuman γ-globulin was present in the serum of virus-infected mice and was in higher titer than in the serum of uninfected mice. However, the antibody was present for only a short period of time and in some experiments was difficult to demonstrate. Further studies showed that the titer of the antibody was reduced to 10 or less following incubation with 2-mercapto-ethanol.

Number of Mice Showing an Immune Response.—The following experiment

was designed to determine how many animals actually showed an accelerated immune response following infection with LDV. Mice were immunized with human γ-globulin and 7 days later a tracer dose of I^{131}–human γ-globulin was given. Elimination of 90% of the tracer in 4 days or less was considered evidence



Text-Fig. 4. Accelerated immune response to human γ-globulin in virus-infected mice Mice were immunized with human γ-globulin on day zero. They were given a tracer dose of the labeled protein (Hu γ-I^{131}) 5, 7, and 9 days later and the rate of antigen elimination was followed. Each group represents the average of 4 animals.

for an immune response. As seen in Table V, 87% of the mice infected with LDV showed an immune response within 9 days after immunization as compared to 4.7% of the uninfected animals. 10 and 11 days postimmunization 100% of the infected mice showed an immune response as compared to 23.8 and 66.6%, respectively, of the uninfected mice. Mice infected with LDV but not immunized with human γ-globulin failed to show any evidence of accelerated antigen elimination.

Relationship of Time of Infection to Immune Response.—In the previous experiments, mice were infected with LDV 1 day prior to immunization. To study the effect of chronic infection on the immune response, animals that had been

TABLE III

Specificity of Immune Response

Immunized*, ‡	Per cent of I^{131} γ-globulin catabolized within 24 hr after injection§	
	Challenged with	
	I^{131}-human γ-globulin	I^{131}-rabbit γ-globulin
None	25.2	19.8
Human γ-globulin	91.3	44.9
Rabbit γ-globulin	27.1	93.7

* Mice were immunized with 300 μg of human or rabbit γ-globulin on day zero and challenged with a tracer dose (20 μg) of the labeled protein 9 days later.

‡ All animals were infected with LDV 1 day prior to immunization.

§ Each group represents the average of 10 animals.

TABLE IV

Hemagglutinating Antibody in Mice Immunized with Human γ-Globulin

Group	Hemagglutination titer				
	Experiment No. 1		Experiment No. 2		Experiment No. 3
	Days after immunization*				
	11	16	12	24	12
Immunized					
Uninfected	20	10	80	20	20
Infected	320	20	640	10	40
Unimmunized					
Uninfected	10	ND‡	20	ND	20
Infected	ND	ND	ND	ND	20

* Groups of 3 to 4 mice from the immune elimination experiments (Figs. 4 to 6) were bled at various times after immunization and the titer of anti-human γ-globulin in the sera was determined.

‡ ND, not done.

infected with LDV 60 days earlier were employed. As seen in Text-fig. 5, the rate of immune elimination in chronically infected animals was considerably faster than in uninfected animals, but was not quite as rapid as in animals that had been infected 1 day prior to immunization. The data in Text-fig. 6 shows

that when animals were infected with LDV 1 or more days after immunization, the accelerated immune response was substantially reduced or not apparent.

Histopathologic Changes in Virus-Infected Mice.—The elevated γ-globulin, the increased level of antibody, the previously demonstrated enlargement of the spleen and lymph nodes (1), and the impairment of the reticuloendothelial system (RES) following infection with LDV (1, 31) led us to examine the spleen and lymph nodes of virus-infected mice for evidence of histopathology. Because of the virtual absence of immunopathology in germfree mice it was thought that histologic changes following virus infection would be more apparent in

TABLE V

*Effect of LDV on the Time of Appearance and the Number of Mice Showing an Immune Response**

Days post-immunization	Unimmunized				Immunized			
	Normal		Infected		Normal		Infected	
	No. IR‡ / No. Mice	% IR§	No. IR / No. Mice	% IR	No. IR / No. Mice	% IR	No. IR / No. Mice	% IR
8	0/18	0	0/17	0	0/21	0	0/23	0
9	0/18	0	0/17	0	1/21	4.7	20/23	87.0
10	0/18	0	0/17	0	5/21	23.8	23/23	100.0
11	0/18	0	0/17	0	14/21	66.6	23/23	100.0

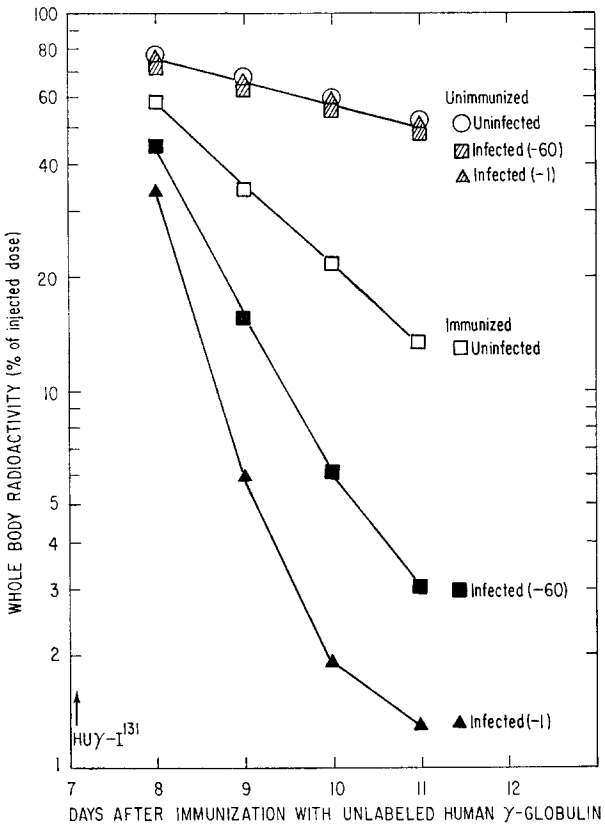
* Mice were infected with LDV on the same day as or 1 day prior to immunization with 300 μg of human γ-globulin. They were challenged 7 days later with approximately 20 μg of I^{131}–human γ-globulin. The disappearance of the tracer was followed daily. Elimination of 90% of the tracer in 4 days or less was considered evidence for an immune response (IR). The day on which the IR occurred was recorded.

‡ Number of mice showing an immune response/total number of mice in the group.

§ Per cent of mice in each group showing an immune response.

these animals. Our studies showed that the splenic white pulp of uninfected germfree animals consisted of densely packed, evenly distributed small lymphocytes and was essentially free of germinal centers (Fig. 1). The red pulp contained no plasma cells. The cortical nodules of the lymph nodes were almost exclusively of the primary type and consisted of closely packed small lymphocytes (Fig. 3). In virus infected mice the spleen and lymph nodes were grossly enlarged. Histologically, the most striking alteration was the development of classical germinal centers. These centers appeared as circular areas of pale-staining cells surrounded by a band of more darkly staining lymphocytes (Figs. 2 and 4). Within the centers were large macrophages with cytoplasmic tingible bodies, mitotic figures, and typical "blast" cells with large, slightly eccentric nuclei and basophilic cytoplasm (Figs. 5 and 6). Mature plasma cells, however, were not observed. The germinal centers appeared within 6 days after

infection but were more advanced and widespread by day 10. At 20 days the splenic white pulp had been obliterated almost completely by the development of germinal centers, and the enveloping collar of small lymphocytes had either disappeared or was very thin. Similarly, the cortical nodules of the lymph



Text-Fig. 5. Accelerated immune response in acutely and chronically infected mice. Numbers in parentheses indicate that the mice were infected 1 or 60 days prior to immunization. Each group represents the average of 10 mice.

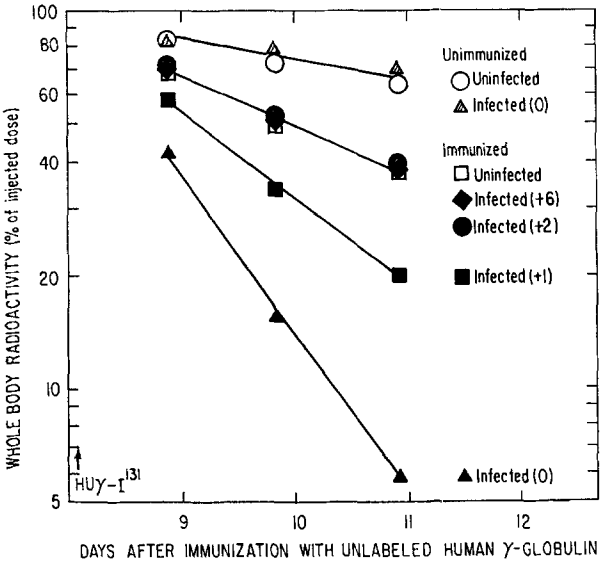
nodes contained large germinal centers and the surrounding lymphocytic band was thin and frequently incomplete.

DISCUSSION

Infection of mice with LDV raised the level of γ-globulin and enhanced the animal's capacity to produce antibody to human γ-globulin. The data suggests that the infectious virus acts as an adjuvant in that it shortens the induction

period, raises the antibody titer, and increases the number of animals showing an immune response.

Antibody to human γ-globulin was detected by both immune elimination and hemagglutination. On the basis of its sensitivity to 2-mercaptoethanol, the hemagglutinating antibody appears to be of the γM-type. The failure to demonstrate hemagglutinating antibody in some experiments and its short survival in others might be related to the rapid catabolism of γM whch is thought to have a half-life of less than 1 day (14–16) and to the postulated



Text-Fig. 6. Effect of LDV on the immune response in animals infected on the same day 0, 1, 2, or 6 days after immunization. Each group represents the average of 10 animals.

cessation of γM-production in the absence of constant antigenic stimulation (14, 17, 18). However, it should be emphasized that mercaptoethanol sensitivity is not a definitive test for the identification of γM-antibody since some properties of γA- and γG- (7S) antibody are also mercaptoethanol sensitive (for references, see reference 19). In fact, by immunoelectrophoresis we were able to show that infection of germfree mice with LDV did raise the level of 7S γ-globulin. In this connection it is pertinent that Benedict (20) could not detect 7S antibody to bovine serum albumin in early primary rabbit antisera if he used the hemagglutination technique, but was able to show 7S antibody if he employed the more sensitive ammonium sulfate salting-out procedure of Farr (21). In our experiments antibody to human γ-globulin was detected by immune elimination long after it was possible to detect antibody by hemagglutination.

In the absence of an adjuvant, it has been shown that immunization of mice by the intravenous or intraperitoneal route is relatively ineffective and under certain circumstances can lead to immunological tolerance rather than antibody formation (10, 22–24). In our experiments, normal mice inoculated intraperitoneally with human γ-globulin did show evidence of an immune response (Table V). The adjuvant effect of LDV greatly enhanced this response. However, the effect of LDV on the immune response following different types, doses, and routes of antigen administration remains to be investigated.

The increase in γ-globulin 6 to 10 days after infection of germfree mice was of particular interest, since neutralizing antibody to LDV was not found in mice infected for as long as 1 month (13) nor in rabbits and guinea pigs given 14 bimonthly injections of the virus (12). Although the elevated γ-globulin might represent nonneutralizing antibody or antibody of low avidity which is directed against the virus or a viral component, the increase in anti–human γ-globulin described above makes it clear that LDV enhances the animal's immune response to a heterologous protein and thus acts as an adjuvant. This points to the possibility that the elevated γ-globulin in the virus-infected germfree animal might represent an increase in so called "natural" antibody or antibody against unrecognized antigens. On the other hand, the virus might simply be stimulating the production of "nonantibody" γ-globulin. Similar problems have been encountered in other systems where the extent of the increase in γ-globulin following the administration of a known antigen and/or adjuvant could not be explained solely on the basis of neutralizing antibody directed against the injected materials (25–30).

Since earlier experiments showed that the clearance of certain proteins (lactic dehydrogenase, isocitric dehydrogenase, etc.) was impaired following infection with LDV (1), we thought that the clearance of endogenous γ-globulin might also be altered. Studies with I^{131}–γ-globulin showed that this was not the case, for infected and uninfected animals catabolized the labeled protein at the same rate. It must be emphasized that only the catabolism of 7S γ-globulin was studied and whether LDV has any effect on the catabolism of other globulins is not known. If the increase in γ-globulin is not due to altered catabolism then the virus must act by increasing the production and/or release of γ-globulin. Since the spleen and lymph nodes contain antibody-producing cells, our attention was directed to the possibility that changes produced in these organs by LDV was affecting the immune response. Several lines of evidence indicated that this was the case. First, these organs were found to be somewhat enlarged following infection with LDV (1, 11, 34). Second, the functional capacity of the RES was markedly altered in virus-infected animals (1, 31). Third, electron microscope studies revealed virus particles within peritoneal macrophages (32), and fourth, tissue culture experiments showed that the virus could grow in cells obtained from the peritoneal cavity (33) or in primary explants from organs

rich in RES cells (1). In the present study we showed that the number of germinal centers was greatly increased following infection with LDV and that the time of appearance of these centers corresponded with the first noticeable rise in γ-globulin. The virtual absence of germinal centers in germfree mice as compared to the large number normally present in conventional mice made it possible to detect and follow the histopathology produced by LDV. Since germinal centers are important sites of antibody production, the increase in the number of these centers in LDV-infected animals could account for the accelerated immune response and the elevated γ-globulin. Studies on the temporal relationship between infection with LDV and injection of the antigen showed that an accelerated immune response occurred if the animals were infected prior to or within 1 day after administration of the antigen, but was greatly reduced or not apparent if animals were infected 2 or more days after administration of the antigen. These preliminary studies suggest that LDV does not produce its adjuvant effect by stimulating antibody synthesis in cells already immunologically committed, but acts by increasing the number of noncommitted cells. A similar mechanism of action has been proposed for other adjuvants (35).

Although an increase in the number of germinal centers appears to be the most likely explanation for the accelerated immune response, other factors also might play a role. First, the level of circulating antibody might be affected if the virus damaged antibody-producing cells so as to release preformed antibody. However, this seems unlikely since, as indicated above, injection of the virus into mice 2 or more days after immunization failed to raise the antibody level in these animals. Second, since macrophages are thought to play a role in initiating antibody production (24, 36–39), the effect of LDV on these cells might affect the immune response. Third, there is evidence in the literature which indicates that nucleic acids may act as an adjuvant (37, 40–45). Since LDV is an RNA virus (4) which is continuously present in the circulation in high titer (3), it is possible that the RNA from the virus or virus-infected cells might contribute to the accelerated immune response.

At the present time, we can do no more than to speculate as to whether the ability of the infected animal to produce an accelerated immune response also increases its resistance to pathogens. Early in the course of infection a decrease in natural resistance would be expected because of the marked impairment of the RES (1, 31). However, the functional capacity of the RES as measured by carbon clearance returns to normal by the end of 1 week (1, 31). The increased antibody-producing capacity of the infected animal might thus outweigh the early deleterious effect of the virus and lead to a state of increased resistance. In another area, viral interference, the accelerated immune response of the LDV-infected animal might also play a role. That is, LDV might interfere with the multiplication of a second virus by accelerating the antibody response

of the host to that virus. In addition, it is not unreasonable to suggest that LDV might affect other immunologic processes such as homograft rejection and autoimmunity. These possibilities are particularly important in light of the fact that many of the widely used transplanted mouse tumors and viruses are contaminated with LDV (1). Lastly, these studies point to the possibility that other viruses also might act as adjuvants.

SUMMARY

Infection of mice with the lactic dehydrogenase virus (LDV) resulted in an elevated level of γ-globulin. Histologic examination of the spleen and lymph nodes revealed that the number of germinal centers was greatly increased. Immunization with human γ-globulin showed that the capacity of the virus-infected animal to produce anti-human γ-globulin was greatly enhanced and that the virus acted as an adjuvant. From these experiments it is concluded that a virus infection (LDV) can affect the immunologic response of the host to a heterologous antigen.

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BIBLIOGRAPHY

1. Notkins, A. L., Lactic dehydrogenase virus, *Bact. Rev.*, 1965, **29,** 143.
2. Trexler, P. C., The use of plastics in the design of isolator systems, *Ann. New York Acad. Sc.*, 1959, **78,** 29.
3. Notkins, A. L., and Shochat, S. J., Studies on the multiplication and the properties of the lactic dehydrogenase agent, *J. Exp. Med.*, 1963, **117,** 735.
4. Notkins, A. L., Recovery of an infectious ribonucleic acid from the lactic dehydrogenase agent by treatment with ether, *Virology*, 1964, **22,** 563.
5. Boyden, S. V., The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera, *J. Exp. Med.*, 1951, **93,** 107.
6. Stavitsky, A. B., Micromethods for the study of proteins and antibodies. I. Procedure and general applications of hemagglutination and hemagglutination-inhibition reactions with tannic acid and protein-treated red blood cells, *J. Immunol.*, 1954, **72,** 360.
7. Deutsch, H. F., and Morton, J. I., Human serum macroglobulins and dissociation units. I. Physicochemical properties, *J. Biol. Chem.*, 1958, **231,** 1107.
8. McFarlane, A. S., Efficient trace-labelling of proteins with iodine, *Nature*, 1958, **182,** 53.
9. Talmage, D. W., Dixon, F. J., Bukantz, S. C., and Dammin, G. J., Antigen elimination from the blood as an early manifestation of the immune response, *J. Immunol.*, 1951, **67,** 243.
10. Dresser, D. W., Elimination of ^{131}I-labelled protein antigens from the circulation of the mouse, *Immunology*, 1960, **3,** 289.
11. Pope, J. H., and Rowe, W. P., Identification of WM1 as LDH virus, and its re-

covery from wild mice in Maryland, *Proc. Soc. Exp. Biol. and Med.*, 1964, **116,** 1015.

12. DuBuy, H. G., and Johnson, M. L., Some properties of the lactic dehydrogenase agent of mice, *J. Exp. Med.*, 1965, **122,** 587.
13. Notkins, A. L., unpublished data.
14. Uhr, J. W., and Finkelstein, M. S., Antibody formation. IV. Formation of rapidly and slowly sedimenting antibodies and immunological memory to bacteriophage ϕX 174, *J. Exp. Med.*, 1963, **117,** 457.
15. Shulman, S., Hubler, L., and Witebsky, E., Antibody response to immunization by different routes, *Science*, 1964, **145,** 815.
16. Fahey, J. L., Sell, S., The immunoglobulins of mice. V. The metabolic (catabolic) properties of five immunoglobulin classes, *J. Exp. Med.*, 1965, **122,** 41.
17. Svehag, S.-E., and Mandel, B., The formation and properties of poliovirus-neutralizing antibody. II. 19S and 7S antibody formation: differences in antigen dose requirement for sustained synthesis, anamnesis, and sensitivity to x-irradiation, *J. Exp. Med.*, 1964, **119,** 21.
18. Möller, G., and Wigzell, H., Antibody synthesis at the cellular level. Antibody-induced suppression of 19S and 7S antibody response, *J. Exp. Med.*, 1965, **121,** 969.
19. Adler, F. L., Studies on mouse antibodies. II. Mercaptoethanol-sensitive 7S antibodies in mouse antisera to protein antigens, *J. Immunol.*, 1965, **95,** 39.
20. Benedict, A. A., Sensitivity of passive haemagglutination for assay of 7S and 19S antibodies in primary rabbit anti-bovine serum albumin sera, *Nature*, 1965, **206,** 1368.
21. Farr, R. S., A quantitative immunochemical measure of the primary interaction between I*BSA and antibody, *J. Infect. Dis.*, 1958, **103,** 239.
22. Dresser, D. W., Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen, *Immunology*, 1962, **5,** 378.
23. Claman, H. N., Tolerance to a protein antigen in adult mice and the effect of non-specific factors, *J. Immunol.*, 1963, **91,** 833.
24. Frei, P. C., Benacerraf, B., and Thorbecke, G. J., Phagocytosis of the antigen, a crucial step in the induction of the primary response, *Proc. Nat. Acad. Sc.*, 1965, **53,** 20.
25. Šterzl, J., Kostka, J., Říha, I., and Mandel, L., Attempts to determine the formation and character of γ-globulin and of natural and immune antibodies in young pigs reared without colostrum, *Folia Microbiol.*, 1960, **5,** 29.
26. Humphrey, J. H., Mechanisms of antibody formation, *in* Proceedings of a Symposium Held in Prague, May 27–31, 1959, (M. Holub and L. Jarošková, editors), Prague, Czechoslovak Academy of Sciences, 1960, 258.
27. Rittenberg, M. B., and Nelson, E. L., Maintenance of globulin levels in x-irradiated rabbits after immunization, *Science*, 1962, **138,** 519.
28. Pernis, B., Cohen, M. W., and Thorbecke, G. J., Specificity of reaction to antigenic stimulation in lymph nodes of immature rabbits. I. Morphologic changes and γ-globulin production following stimulation with diphtheria toxoid and silica, *J. Immunol.*, 1963, **91,** 541.
29. Silverstein, A. M., Thorbecke, G. J., Kraner, K. L., and Lukes, R. J., Fetal re-

sponse to antigenic stimulus. III. γ-Globulin production in normal and stimulated fetal lambs, *J. Immunol.*, 1963, **91,** 384.

30. Humphrey, J. H., The nonspecific globulin response to Freund's adjuvant, *in* Colloques Internationaux du Centre National de la Rechérche Scientifique, Tolérance Acquise et Tolérance Naturelle a l'égard de Substances Antigéniques Définies, Paris, Centre National de la Rechérche Scientifique, 1963, 401.
31. Mahy, B. W. J., Action of Riley's plasma enzyme-elevating virus in mice, *Virology*, 1964, **24,** 481.
32. De-Thé, G., and Notkins, A. L., Ultrastructure of the lactic dehydrogenase virus (LDV) and cell-virus relationships, *Virology*, 1965, **26,** 512.
33. Evans, R., Replication of Riley's plasma enzyme elevating virus in vitro, *J. Gen. Microbiol.*, 1964, **37,** vii.
34. Pope, J. H., Studies of a virus isolated from a wild house mouse, *Mus musculus*, and producing splenomegaly and lymph node enlargement in mice, *Australian J. Exp. Biol. and Med. Sc.*, 1961, **39,** 521.
35. Munoz, J., Effect of bacteria and bacterial products on antibody response, *Advances Immunol.*, 1964, **4,** 397.
36. Fishman, M., Antibody formation in vitro, *J. Exp. Med.*, 1961, **114,** 837.
37. Fishman, M., and Adler, F. L., Antibody formation initiated in vitro. II. Antibody synthesis in x-irradiated recipients of diffusion chambers containing nucleic acid derived from macrophages incubated with antigen, *J. Exp. Med.*, 1963, **117,** 595.
38. Mollo, F., Governa, M., Citati, C., and Dellepiane, M., Experimental observations on the participation of macrophages in the mechanism of antibody production, *Experientia*, 1963, **19,** 582.
39. Uhr, J. W., and Weissmann, G., Intracellular distribution and degradation of bacteriophage in mammalian tissues, *J. Immunol.*, 1965, **94,** 544.
40. Šterzl, J., and Hrubešová, M., The transfer of antibody formation by means of nucleoprotein fractions to non-immunized recipients, *Folia Biol., (Prague)*, 1956, **2,** 21.
41. Garvey, J. S., and Campbell, D. H., The retention of S^{35}-labelled bovine serum albumin in normal and immunized rabbit liver tissue, *J. Exp. Med.*, 1957, **105,** 361.
42. Hrubešová, M., Askonas, B. A., and Humphrey, J. H., Serum antibody and γ-globulin in baby rabbits after transfer of ribonucleoprotein from adult rabbits, *Nature*, 1959, **183,** 97.
43. Taliaferro, W. H., and Jaroslow, B. N., The restoration of hemolysin formation in x-rayed rabbits by nucleic acid derivatives and antagonists of nucleic acid synthesis, *J. Infect. Dis.*, 1960, **107,** 341.
44. Merritt, K., and Johnson, A. G., Studies on the adjuvant action of bacterial endotoxins on antibody formation. VI. Enhancement of antibody formation by nucleic acids, *J. Immunol.*, 1965, **94,** 416.
45. Braun, W., and Nakano, M., Influence of oligodeoxyribonucleotides on early events in antibody formation, *Proc. Soc. Exp. Biol. and Med.*, 1965, **119,** 701.

EXPLANATION OF PLATE 50

Fig. 1. Spleen from a germfree mouse. White pulp (*wp*) appears uniformly dark owing to the large number of small lymphocytes and the absence of germinal centers. Hematoxylin and eosin. × 25.

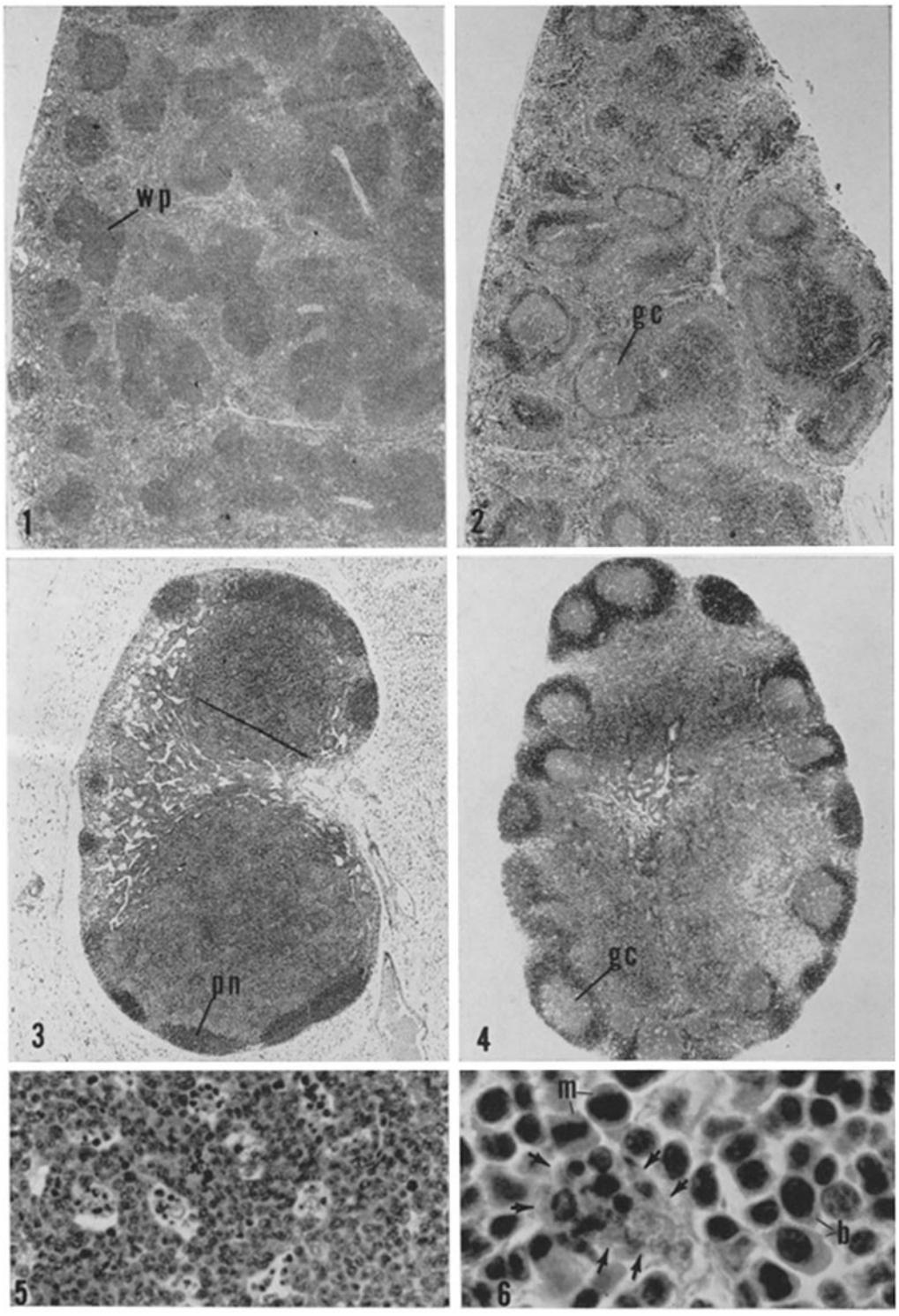
Fig. 2. Spleen from a germfree mouse 20 days post LDV. Within the white pulp are many large pale-staining germinal centers (*gc*) which are surrounded by a thin band of darkly staining lymphocytes. Hematoxylin and eosin. × 25.

Fig. 3. Lymph node from a germfree mouse showing primary nodules (*pn*). Germinal centers are absent. Hematoxylin and eosin. × 25.

Fig. 4. Lymph node from a germfree mouse 20 days post LDV. Large germinal centers (*gc*) are present throughout the cortex. Hematoxylin and eosin. × 25.

Fig. 5. Higher magnification of a germinal center from Fig. 2 showing macrophages with cytoplasmic tingible bodies. Hematoxylin and eosin. × 293.

Fig. 6. Portion of a geminal center from the spleen of an LDV-infected mouse showing mitotic figures (*m*), "blast" cells (*b*), and a large macrophage (arrows) with cytoplasmic tingible bodies. Hematoxylin and eosin. × 1010.



(Notkins et al.: γ-Globulin and antibody production)