Immunology 1983 **48** 511

Antibody classes and subclasses in circulating immune complexes isolated from mice infected with lactic dehydrogenase virus

T. L. McDONALD, T. DONNELLY, ANNIKA WEBER & LINDA QUENETTE *University of Nebraska Medical Center, Omaha, Nebraska, U.S.A.*

Accepted for publication 23 August 1982

Summary. A chronological study of circulating immune complexes (CIC) in lactic dehydrogenase virus (LDV)-infected mice has been performed. The results demonstrate that CIC containing immunoglobulin of the IgM class were isolated between days 3 and 9 post-LDV infection and corresponded to an increase in serum IgM. IgG1-containing CIC were also transient in the serum of LDV-infected mice in that they were isolated only between days 5 and 13. The occurrence of IgG1 CIC did correlate with an increase in total IgG1 in the serum, however, it did correlate with a small (1:10) increase in IgG1 anti-LDV activity. In contrast, CIC containing immunoglobulin of the serum IgG2 subclass were not isolated from LDV-infected serum until 15 days post infection. This chronological appearance of IgG2 CIC did not correlate with the observed increase in total IgG2 concentration in LDV-infected mice on day 7, however, was analogous to the rapid increase in free serum LDV-specific antibody. We propose that the non-specific suppression of the immune response and tumour enhancement during the acute phase of LDV infection could be due to the immunoregulatory properties of IgG1 CIC.

Abbreviations: LDV, lactic dehydrogenase virus; IC, immune complexes; CIC, circulating immune complex; LDH, lactic dehydrogenase; Clq LF, Clq-like factor; TNE, Tris-sodium chloride-ethylene diamine tetraacetic acid buffer; RIEP, rocket immunoelectrophoresis; ELISA, enzyme-linked immunosorbent assay.

Correspondence: Dr T. L. McDonald, University of Nebraska Medical Center, Department of Medical Microbiology, 42nd and Dewey Avenue, Omaha, Ne. 68105, U.S.A.

0014-2805/83/0300-0511$02.00

INTRODUCTION

Lactic dehydrogenase virus (LDV) is a small enveloped RNA virus that causes a persistent viraemia in infected mice. Virus–antibody complexes have been identified in the serum of these infected mice throughout the animals' life (McDonald, 1982; Notkins, Mahar, Scheele & Goffman, 1966; Riley, 1974; Riley & Spackman, 1974), however, immune complex (IC) deposits and histological alteration of the glomeruli are minimal (Oldstone & Dixon, 1971; Porter & Porter, 1971).

In dual-infected animal models, LDV has been reported to enhance transplantable tumour growth (Howard, Notkins & Mergenhagen, 1969; Isakov, Feldman & Segal, 1981; McDonald, 1982; Riley & Spackman, 1974; Turner, Bert, Bassin, Spahn & Chirigos, 1971), alter the growth of 3-methylcholanthrene-induced fibrosarcoma (Henderson, Chang & Turk, 1979) exacerbate murine malaria (Henderson, Tosta & Wedderburn, 1978) decrease resistance to infection by *Listeria monocytogenes* (Bonevtre, Bubel, Michael & Nickol, 1980), alter autoimmune responses in NZB mice (Oldstone & Dixon, 1972) and influence the antibody response to T-independent and

T-dependent antigens (Michaelides & Simms, 1977a, 1980). These effects observed in dual-infected animals are dependent upon the time interval which elapses between LDV infection and inoculation of the second agent. Enhancement effects in dual-infected animals appear to be restricted to the acute phase of LDV infection and have attributed to suppression of T-cell-dependent function (Howard *et al.*, 1969; Michaelides & Schlesinger, 1973; Michaelides & Simms, 1980; Turner *et al.*, 1971) and/or inhibition of macrophage function by LDV (Mahy, Rowson, Parr & Salomon, 1965). However, the fact that the enhancement effects are transient in spite of the continuing high virus titres in the blood remains an unresolved paradox.

Data generated from studies concerning cell-mediated immune blocking factors and enhancement in persistent virus-infected animal models and cancer patients have suggested that the mediator of such phenomena is the immune complexes (IC) (Hellstrom & Hellstrom, 1974; Sjorgen, Hellstrom, Bansal & Hellstrom, 1971). It is also apparent that the immunoglobulin composition of the IC is an important factor in determining its functional properties.

Recent reports concerning the immunoregulatory role of IgG antibody subclasses and IC show that IC containing IgG1 and to a lesser extent IgG2 enhance allografted tumours (Harris, Harris, Henri & Farber, 1978; Voisin, 1980). Duc, Kinsky, Kanellopoulos & Voisin, 1975), however, reported that by complexing IgG1 and IgG2 immunoglobulins from anti-A/J immune serum to A/J soluble H-2^a antigens, IgG2-containing IC were enhancing. Serum antibody or antigen alone were not enhancing which further demonstrates the importance of immune complexes in immunoregulatory mechanisms originally suggested by Hellstrom & Hellstrom (1974).

LDV infection has an adjuvant-like effect in mice that appears to be restricted to the IgG2a subclass of immunoglobulins whereas serum levels of IgG1 remain unchanged (Michaelides & Simms, 1977b). The proposed role of IC immunoregulation (Hellstrom & Hellstrom, 1974; Sinclair, 1978; Voisin 1980), and the importance of immunoglobulin class and subclass in the mechanism of action of these IC suggests that the enhancing effects observed in LDV-infected mice might involve immunoregulatory properties of LDV–antibody complexes.

It was the purpose of this chronological study to isolate IC from the serum of LDV-infected mice and determine the concentration of immunoglobulin content with respect to class and subclass of antibody.

MATERIALS AND METHODS

Virus

Frozen LDV-infected mouse serum was originally obtained from Dr D. Burger, Washington State University (Pullman, Wash.) and used as seed virus. Stock virus preparations, titrations and storage were performed as described elsewhere (Rowson & Mahy, 1957).

Mice

BALB/c mice (Charles Rivers Labs, Wilmington, Mass.) were caged in ten groups of seven each and infected with 0·1 ml of stock LDV by intraperitoneal injection. Chronological samples were obtained daily by pooling approximately 0·5 ml of blood, obtained by retro-orbital sinus puncture, from all mice in one cage. The serum collected following low speed centrifugation was assayed immediately for lactic dehydrogenase activity (LDH) and the remaining serum pool was frozen at $-70°$ in 0·5 ml aliquots.

Using this method, each group of mice was bled every 10 days and all ten cages were maintained throughout one 33-day chronological study. Alternate daily bleedings of three cages of seven uninoculated BALB/c mice were used for normal mouse serum pools. All uninoculated animals were housed separately from LDV mice.

Isolation of circulating immune complexes from serum

Circulating immune complexes (CIC) were isolated from chronological serum samples from LDV-infected mice as described by McDonald (1981). Equal volumes (200 μl) of the equine Clq-like factor (Clq-LF; 1 mg/ml) and mouse serum were mixed in polystyrene tubes and incubated for 15 min at 37°. The contents of the tubes were removed and dialysed against 2 litres 0·05 M Tris-HCl, pH 8·1 for 1 hr. The material was then centrifuged at 1200 ***g*** for 5 min and the precipitate resuspended and washed twice in the dialysing buffer. The final precipitate was dissolved in 200 μl of 0·05 M Tris-HCl, pH 8·1, containing 0·5 M NaCl and analysed for mouse IgG content by rocket immunoelectrophoresis. When necessary, all serum samples and IC isolates were stored at $-70°$.

Rocket immunoelectrophoresis

A modification of the rocket immunoelectrophoresis (RIEP) originally described by Laurell (1972) was used for the quantification of immunoglobulin classes and subclasses in both the original serum samples and

the Clq-like factor precipitates. Before gel casting, the appropriate antiserum with specificity to mouse immunoglobulin (Research Prod. Int., Elk Grove Village, Ill.) was diluted to 1% v/v final concentration in 56° agarose (1% w/v in barbital buffer, pH 8·6; Laurell, 1972). All serum samples diluted 1:40 in 0·05 M phosphate-buffered saline, pH 7·2, and undiluted IC isolates were incubated 30 min at 37° with an equal volume of 1% formaldehyde before RIEP. Five microlitre aliquots were then applied to 3 mm diameter wells, cut at the base of the gel slab, and immediately electrophoresed at 5 V/cm gel for 4 hr at 4°.

Five microlitres samples of IgM, IgG1 and IgG2 (Research Prod. Int, Elk Grove Village, Ill.) were used as standards at concentrations of 200, 100, 50 and 25 μg/ml.

Following RIEP, the gel plates were dried and stained with 0·05% w/v Coomassie brilliant blue R-250 and destained with ETOH, H_2O, glacial acetic acid (4·5:4·5:1·0) as described by Laurell (1972).

Enzyme-linked immunosorbent assay (ELISA)

An indirect ELISA method described by Voller, Bidwell & Bartlett (1976) was used to quantify anti-LDV activity in the chronological serum samples obtained from LDV-infected mice. The wells of polystyrene substrate plates (Dynatech Laboratories, Inc., Alexandria, Va.) were sensitized with 0·1 ml of purified LDV (Michaelides & Schlesinger, 1974) which contained 1 μg of protein and a titre of 1×10^5 mouse infective dose. Test samples were incubated in antigen-coated wells and analysed for bound mouse IgG1 or IgG2 by further incubation with the appropriate alkaline phosphate-conjugated (Sigma Chemical Co., St. Louis, Mo.) rabbit anti-mouse immunoglobulin subclass (Research Prod. Int., Elk Grove Village, Ill.).

LDV antigen

LDV used for the ELISA test antigen was purified from a 50 ml pool of 48 hr viraemic mouse serum by sucrose density gradient fractionation as previously described (Michaelides & Schlesinger, 1974). Purified LDV was titrated (Rowson & Mahy, 1975) and diluted to 1×10^6 mouse infection units/ml. The protein concentration of the ELISA-coating antigen was 10 mg protein/ml.

Protein determinations

Protein concentrations were estimated by calculating the ratios of the absorbances at 224 and 233 nm according to the method of Groves, Paves & Sells (1967). The protein standard was Cohn Fraction II human gamma globulins (Sigma Chemical Co., St. Louis, Mo.).

RESULTS

LDV infection

Immediately following separation, the serum samples were analysed for lactic dehydrogenase (LDH) activity in order to establish LDV infection in the experimental mice. The LDH activity of the serum samples throughout this chronological study demonstrated a typical pattern for LDV infection. An increase in LDH activity to ten times above normal was observed at 96 hr in the serum of LDV-infected mice (data not shown). This activity decreased over the next 3 days to five times normal LDH levels and remained at that activity for the remainder of this chronological study.

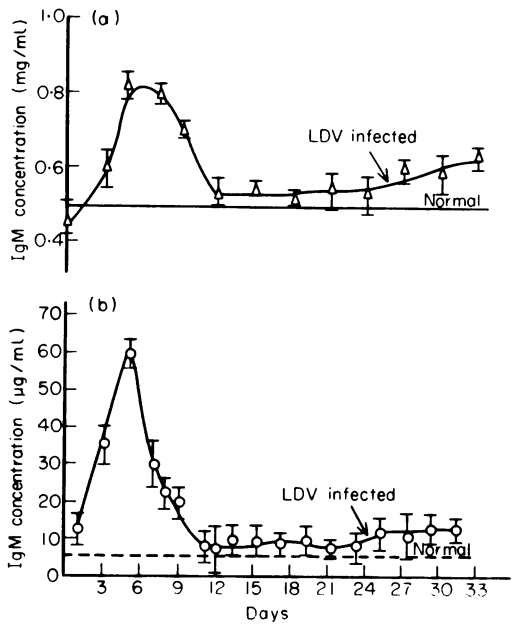

Figure 1. Chronological appearance and serum concentrations (mean ± SEM) of IgM and IgM-containing immune complexes in LDV-infected mice. (a) Pooled serum samples were diluted 1:60 in 0·05 M phosphate-buffered saline, pH 7·2 and quantified for IgM concentration by RIEP as described in Material and methods. (b) CIC were isolated from LDV infected and normal mouse serum pools by co-precipitation with equine Clq-LF and analysed for IgM content by RIEP as described in Materials and Methods.

Isolation of CIC

The reproducibility of Clq-LF in isolating CIC from LDV-infected and normal mice was evaluated by analysis of frozen aliquots of each pooled serum sample at three different times. The greatest standard error of the mean concentration of CIC in these triplicate serum samples was ± 6 μg/ml (range ± 2 to 6 μg) analysis of individual samples run to triplicate, in order to assess the reproducibility of the RIEP for analysis of CIC concentration, demonstrated a standard error of ± 4 μg/ml. This analysis was convincing that the test system was highly reproducible for both the isolation of CIC from serum and the analysis for immunoglobulin content.

The sensitivity of the RIEP was determined by electrophoresing various concentrations of each immunoglobulin class or subclass against the appropriate homologous antiserum-containing gel. The lowest detectable concentration was 5 μg/ml for IgM and 10 μg/ml for all IgG subclasses.

Serum and CIC immunoglobulins

A chronological study of the serum concentration of IgM in normal and LDV-infected mice was performed and the results are shown in Fig. 1a. A rapid increase in serum IgM concentrations occurred between 3 and 5 days post LDV infection. This elevated level of serum IgM was transient as a decrease was observed starting at day 7 and continuing through day 12 to levels slightly higher than those observed in non-infected mice. It is of interest that between days 24 and 33, and the serum IgM concentration of LDV-infected mice appeared to be elevated, however, the rate of increase and the concentration were not as marked as the initial changes observed between days 3 and 9. Since quantification was not performed beyond day 33 it is not known if this secondary increase of IgM in the serum of LDV-infected animals was also transient.

The concentration of IgM-containing CIC that were isolated from these serum samples is shown in Fig. 1b. CIC that contained IgM antibody were isolated only between 3 and 9 days post-LDV infection with a maximum concentration of 60 μg/ml serum on day 6. The slight elevation in isolated CIC between days 25 and 33 corresponded to the increase in serum IgM (Fig. 1a) during that time.

Further analysis of these 33-day chronological sera from LDV-infected mice for changes in the concentration of IgG2 was performed and the data expressed in Fig. 2a. The earliest detectable increase in serum IgG2

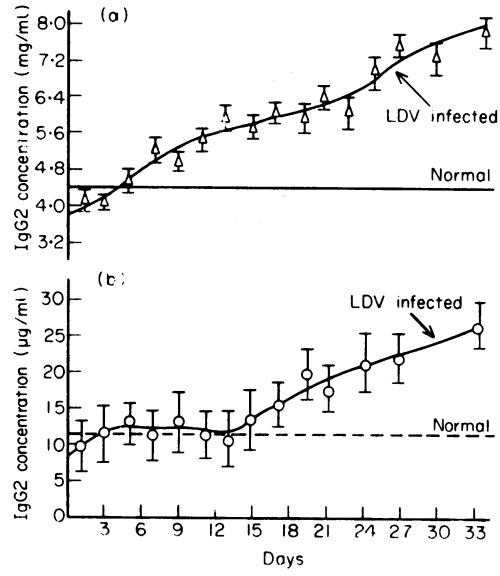

Figure 2. Chronological appearance and serum concentrations (mean ± SEM) of IgG2 and IgG2-containing immune complexes in LDV-infected mice. (a) Pooled serum samples were diluted 1:80 in 0·05 M phosphate-buffered saline, pH 7·2 and quantified for IgG1 concentration by RIEP as described in Materials and Methods. (b) CIC were isolated from LDV-infected normal serum pools by co-precipitation with equine Clq-LF and analysed for IgG2 content by RIEP as described in Materials and Methods.

concentration in LDV-infected mice was observed on day 7. From days 7 to 33, the IgG2 serum concentration continued to increase from 4·5 mg/ml to a maximum of 8·0 mg/ml. The data also showed that this chronological increase in serum IgG2 was gradual over the entire assay period.

When CIC, isolated during this chronological study, were analysed for IgG2 content, it was observed that no IgG2-containing CIC were isolated from the sera of LDV-infected mice during the first 15 days post LDV infection (Fig. 2b). However, from days 17 to 33, CIC that contained IgG2 were isolated in concentrations ranging from 12 μg/ml on day 15 to 30 μg/ml on day 33. Although the serum concentration of IgG2 increased from 4·5 mg/ml to 5·7 mg/ml during the first days post LDV infections, it is of interest that no IgG2-containing CIC were isolated with Clq-LF.

The concentrations of IgG1 in the chronological serum samples from LDV-infected mice were not different from those concentrations in normal mouse serum until day 18 (Fig. 3a). This increase was not as

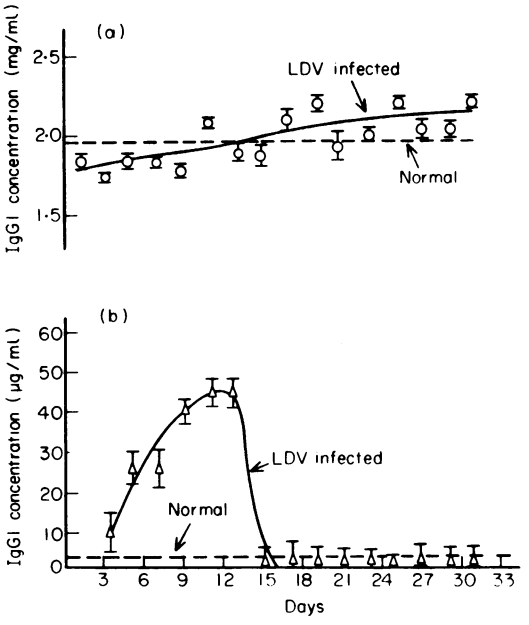

Figure 3. Chronological appearance and serum concentrations (mean ± SEM) of IgG1 and IgG1-containing immune complexes in LDV-infected mice. (a) Pooled serum samples were diluted 1:40 in 0·05 M phosphate-buffered saline, pH 7·2 and quantified for IgG1 concentration by RIEP as described in Materials and Methods. (b) CIC were isolated from LDV-infected and normal serum pools by co-precipitation with equine Clq-LF and analysed for IgG2 content by RIEP as described in Materials and Methods.

marked as previously observed with IgM and IgG2 in that only a 200 µg/ml increase above normal was observed at the termination of this chronological study.

The data generated from analysis of the IgG1 concentration of CIC isolated from the sera of LDV-infected mice are represented in Fig. 3b. Interestingly, IgG1-containing CIC were isolated from LDV-infected sera only between days 5 and 13 post LDV infection. The concentration of IgG1 in these isolated CIC ranged from approximately 22 µg/ml serum on day 6 to approximately 42 µg/ml serum on day 13 of LDV infection.

No increase in serum IgG1 was detected during the chronological period in which IgG1 CIC were isolated from the serum in LDV-infected mice. Conversely, when detectable increases of serum IgG1 occurred in the LDV-infected mice after day 15, no CIC containing IgG1 were isolated.

Antibody specificity

The purpose of these experiments was to determine the specificity of the antibody moiety in the isolated CIC and also to determine if the increase in serum IgG in LDV-infected mice was representative of anti-LDV activity.

In the first experiments, diluted samples containing isolated CIC were incubated in ELISA wells that were previously coated with purified LDV antigen and the data scored for anti-LDV activity following addition of alkaline phosphatase-conjugated anti-mouse IgG as the ELISA second antibody. No anti-LDV activity was detected in any of the samples containing isolated CIC (data not shown). This indicates that the LDV-specific antibody demonstrated in the isolated CIC by RIEP was bound to the LDV antigen and was not available for further antigen binding in the ELISA.

The results from additional experiments designed to quantify free anti-LDV antibody in the serum of LDV-infected mice were illustrated in Fig. 4. In these experiments, the LDV-specific antibody was further characterized relative to its subclass by employing the appropriate alkaline phosphatase-conjugated second antibody in the ELISA.

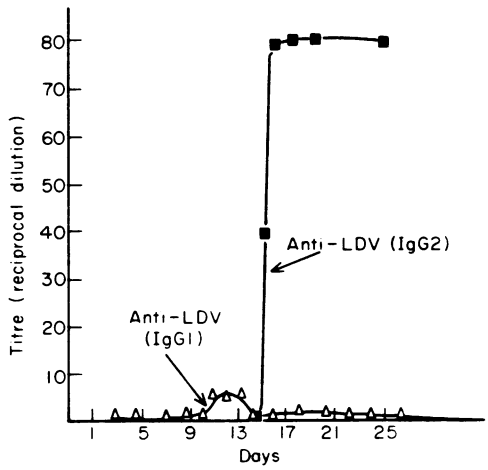

Figure 4. Chronological appearance and ELISA titres of mouse IgG1 and IgG2 antibody with specificity for LDV. One hundred microlitres of two-fold dilutions of pooled serum samples from LDV-infected and normal mice were incubated in LDV-coated ELISA plates for 1 hr at 40°. Rabbit anti-mouse IgG1 or IgG2 conjugated to alkaline phosphatase was used as the second antibody. Anti-LDV titre are scored as the reciprocal of the serum dilution.

Two peaks of serum anti-LDV antibody were detected in samples obtained from LDV-infected mice during this chronological study. The first appearance of free anti-LDV activity was detected in serum samples between days 9 and 13. A maximum anti-LDV titre of 1:10 was observed in these samples and all of the activity was associated with the IgG1 subclass of IgG.

Serum anti-LDV activity of IgG2 subclass specificity was not detected in LDV-infected mice until day 15 (Fig. 4). The IgG2 anti-LDV titre increased rapidly to 1:80 on day 17 and remained at that activity for the duration of this chronological study.

DISCUSSION

A method for the isolation of CIC by an equine Clq-like factor, recently reported by McDonald (1981) has been successfully used to isolate CIC from the serum of LDV-infected mice. Although the mechanism by which Clq-LF isolates CIC, or the efficiency of binding complexed immunoglobulins of different classes and subclasses was not determined by McDonald (1982), it has been our experience that this Clq-LF co-precipitates IgM, IgG1, and IgG2 immunoglobulin-containing CIC from LDV-infected mouse serum.

The isolation of CIC containing antibodies of the IgM class early in LDV-infection corresponded to an increase in serum IgM. These IgM-containing CIC were cleared from the blood by day 10 as evidenced by the failure to co-precipitate them from LDV-infected serum by Clq-LF.

The increase in IgG2 in the serum of LDV-infected mice between days 6 and 13 was not coincidental with an increase in IgG2-containing CIC. CIC containing IgG2 were not isolated until day 15 at which time the IgG2 concentration in LDV-infected mouse serum had significantly increased. It is possible that this increase in IgG2 in the serum of LDV-infected mice may not all be accounted for by increased anti-LDV activity since results have demonstrated that acute LDV infection can have an adjuvant-like effect with T-dependent antigens (Michaelides & Sims, 1977b). However, it is unlikely that IgG2-containing CIC are absent from the serum of LDV-infected mice between days 6 and 13 post infection and Clq-LF could bind CIC-containing IgG2 subclass of antibody at a lower efficiency than IgG1 and IgM.

This failure to isolate CIC containing antibodies of the IgG2 subclass during serum IgG2 elevation can be explained by limitations of the CIC isolation procedure. It has recently been reported that Clq-LF co-precipitated IC from serum most effectively when antigen and antibody concentrations were at equivalence or antibody excess (McDonald, 1982). Therefore, IgG2 subclass CIC formed between day 6 and 13 post LDV infection could have been in antigen excess and equivalence or antibody excess did not occur until day 15. This hypothesis is supported by the fact that free anti-LDV antibody activity of IgG2 subclass was not detected in serum from LDV-infected mice until day 15. Since free antibody to LDV would not be detected in serum until all available antigenetic determinants were saturated, it is likely that anti-LDV of IgG2 subclass in circulation before 13 days post LDV infection is bound as a CIC in antigen excess.

In contrast to IgG2, CIC containing antibodies of the IgG1 subclass were isolated from LDV-infected serum by Clq-LF only between days 6 and 13 post LDV infection. This would indicate that IgG1 antibody to LDV approached ag/ab equivalence in the serum earlier than IgG2 antibody. Supportive data generated in the ELISA study of serum anti-LDV activity demonstrated that free anti-LDV antibody of IgG1 subclass occurred only between days 9 and 13. It would appear that since the total IgG1 concentration in LDV-infected mice did not increase above normal levels during this time period and the free anti-LDV activity in the serum was titred at only 1:10 by ELISA, IgG1 antibody to LDV is essentially all bound as a virus-antibody complex. Moreover, this data clearly demonstrated that the occurrence of CIC of subclass IgG1 in LDV-infected mice is transient, similar to CIC of IgM class antibody and suggests LDV-induced suppression of IgG1 antibody synthesis. This observation is consistent with those of Michaelides and Simms who suggested that the hyperglobulinaemia in LDV-infected BALB/c mice represents anti-LDV antibody that is clonally restricted to IgG2 subclass and not IgG1 (Michaelides & Simms, 1977).

In summary, LDV has been reported to enhance tumour growth (Howard *et al.*, 1969; Isakov *et al.*, 1981; Michaelides & Schlesinger, 1973; Riley & Spackman, 1974; Turner *et al.*, 1980), impair resistance to bacterial infections (Bonventre *et al.*, 1980), and exacerbate murine malaria (Henderson *et al.*, 1978) in dual-infected animals models. The data expressed herein and recent reports of the immunoregulatory potential of IgG1-containing IC (Sinclair, 1978; Voisin, 1980), suggests that the immunoregulatory

potential of LDV infection on the inhibition of T-cell-dependent reactions may be related to the appearance and duration of IgG1 antibody subclass of CIC in these animals.

ACKNOWLEDGMENT

This work was supported by PHS Grant 5 RO1 CA 28205-02 awarded by the National Cancer Institute, PHHS.

REFERENCES

BOVENTRE P.F., BUBEL H.C., MICHAEL J.G. & NICKOL A.D. (1980) Impaired resistance to bacterial infection after tumor implant is traced to lactic dehydrogenase virus. *Infect. Immun.* **30,** 316.

DUC H.T., KINSKY R.G., KANELLOPOULOS J. & VOISIN G.A. (1975) Biological properties of transplantation immune sera. IV. Influence of the course of immunization, dilution and complexing to antigen on enhancing activity of Ig classes. *J. Immunol.* **115,** 1143.

GROVES W.E., DAVIS C.F. JR. & SELLS B.H. (1967) Spectrophotometric determination of microgram quantities of protein without nucleic acid and interference. *Anal. Biochem.* **22,** 195.

HARRIS T.N., HARRIS S., HENRI E.M. & FARBER M.B. (1978) Enhancement of growth of allogeneic mouse tumor by the IgG1 fraction of alloantibody preparations. *J. natn. Cancer Inst.* **60,** 167.

HELLSTROM K.E. & HELLSTROM I. (1974) Lymphocyte-mediated cytotoxicity and blocking serum to tumor antigens. *Adv. Immunol.* **18,** 209.

HENDERSON D.C., CHANG R.W.S. & TURK J.L. (1979) Infection with LDH virus alters host response to tumors. *Br. J. Cancer,* **39,** 453.

HENDERSON D.C., TOSTA C.E. & WEDDERBURN NINA (1978) Exacerbation of murine malaria by concurrent infection with lactic dehydrogenase-elevating virus. *Clin. exp. Immunol.* **33,** 357.

HOWARD R.J., NOTKINS A.L. & MERGENTHAGEN S.E. (1969) Inhibition of cellular immune reactions in mice infected with lactic dehydrogenase virus. *Nature (Lond.),* **221,** 873.

ISAKOV N., FELDMAN M. & SEGAL S. (1981) Effect of lactic dehydrogenase virus infection on tumor induction and tumor growth. *Cancer Res.* **41,** 667.

LAURELL C. (1972) Electroimmuno assay. *Scand. J. Clin. Invest.* **29,** 21.

MCDONALD T.L. (1981) Isolation of immune complexes by an equine Clq-like factor. *Immunol. Comm.* **45,** 741.

MCDONALD T.L. (1982) Isolation of Clq-binding virus-antibody immune complexes from lactic dehydrogenase virus (LDV)-infected mice. *Immunology,* **45,** 365.

MAHY B.W.J., ROWSON K.E.K., PARR C.W. & SALAMAN M.H. (1965) Studies on the mechanism of action of Riley virus. I. Action of substances affecting the reticuloendothelial system on plasma enzyme levels in mice. *J. exp. Med.* **122,** 967.

MICHAELIDES M.C. & SCHLESINGER S. (1973) Structural proteins of lactic dehydrogenase virus. *Virology,* **55,** 211.

MICHAELIDES M.C. & SCHLESINGER S. (1974) Effect of acute or chronic infection with lactic dehydrogenase virus (LDV) on the susceptibility of mice to plasmacytoma MOPC-315. *J. Immunol.* **112,** 1560.

MICHAELIDES M.C. & SIMMS E.S. (1977a) Immune responses in mice infected with lactic dehydrogenase virus. II. Contact sensitization to DNFB and characterization of lymphoid cells during acute LDV infection. *Cell. Immunol.* **29,** 285.

MICHAELIDES M.C. & SIMMS E.S. (1977b) Immune responses in mice infected with lactic dehydrogenase virus. I. Antibody response to DNP-BGG and hyperglobulinaemia in BALB/c mice. *Immunol.* **32,** 981.

MICHAELIDES M.C. & SIMMS E.S. (1980) Immune responses in mice infected with lactic dehydrogenase virus. III. Antibody response to a T-dependent and a T-independent antigen during acute and chronic LDV infection. *Cell Immunol.* **50,** 253.

NOTKINS A.L., MAHAR S., SCHEELE C.S. & GOFFMAN J. (1966) Infectious virus-antibody complex in the blood of chronically infected mice. *J. exp. Med.* **124,** 81.

OLDSTONE M.B.A. & DIXON F.J. (1971) Lactic dehydrogenase virus-induced immune complex type of glomerulonephritis. *Immunology,* **106,** 1260.

OLDSTONE M.B.A. & DIXON F.J. (1972) Inhibition of antibodies to nuclear antigen and to DNA in New Zealand mice infected with lactic dehydrogenase virus. *Science,* **175,** 784.

PORTER D.D. & PORTER H.G. (1971) Deposition of immune complexes in the kidneys of mice infected with lactic dehydrogenase virus. *J. Immunol.* **106,** 1264.

RILEY V. (1974) Persistence and other characteristics of the lactic dehydrogenase-elevating virus (LDH-virus). *Progr. Med. Virol.* **18,** 198.

RILEY V. & SPACKMAN D. (1974) Modifying effects of a benign virus on the malignant process and the role of physiological stress on tumor incidence. *Fogarty Int. Centre Proc.* **28,** 319.

ROWSON K.E.K. & MAHY B.W.J. (1975) Lactic dehydrogenase virus. In: *Virology Monographs,* Vol. 13. (Ed. by S. Gard and C. Hallover), Springer-Verlag, New York.

SINCLAIR N.R. STC. (1978) Immunoregulation by antibody and antigen-antibody complexes. *Transplantn Proc.* **10,** 349.

SJOGREN H.O., HELLSTROM I., BANSAL S.C. & HELLSTROM K.E. (1971) Suggestive evidence that the 'blocking antibodies' of tumor-bearing individuals may be antigen–antibody complexes. *Proc. natn. Acad. Sci.* **68,** 1372.

TURNER W., BERT P.S., BASSIN R., SPAHN G. & CHIRIGOS M.A. (1971) Potentiation of murine sarcoma virus (Harvey) (Moloney) oncogenicity in lactic dehydrogenase-elevating virus-infected mice. *Exp. Biol. Med. Proc.* **136,** 1314.

VOISIN G.A. (1980) Role of antibody classes in the regulatory facilitation reaction. *Immunol. Rev.* **3,** 59.

VOLLER A., BIDWELL D. & BARTLETT A. (1976) Microplate enzyme immunoassays for immunodiagnosis of virus infections. In: *Manual of Clinical Immunology* (Ed. by N. Rose and H. Friedman), pp. 506–512. American Society for Microbiology, Washington, D.C.