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Inhibition of diabetes in BB rats by virus infection

II. EFFECT OF VIRUS INFECTION ON THE IMMUNE RESPONSE TO NON-VIRAL AND VIRAL ANTIGENS

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SUMMARY

Lymphocytic choriomeningitis virus (LCMV) infection prevents the usual insulin-dependent diabetes mellitus of aged BB rats (Dyrberg, Schwimmbeck & Oldstone, 1988; Schwimmbeck, Dyrberg & Oldstone, 1988). In this study earlier observations are extended by noting that LCMV infection substantially alters the immune responses of BB diabetic-prone (dp) rats. The control, uninfected rats make vigorous primary and secondary antibody responses when challenged with keyhole limpet haemocyanin (KLH), human immunoglobulin (HuIg) or sheep red blood cells (SRBC). Such rats fail to mount a primary response to bovine serum albumin (BSA) but do produce a moderate secondary response. They mount good antibody responses to LCMV but fail to generate either primary or secondary LCMV-specific cytotoxic T-lymphocyte (CTL) responses or CTL responses to Pichinde virus. In contrast, BB dp rats acutely infected with LCMV generate no primary immune responses to SRBC, KLH or BSA and only meager responses when challenged with HuIg. They mount secondary reponses to KLH, HuIg and BSA that approximate those of their uninfected litter mates, but have a comparatively lower response to SRBC. LCMV binds to and infects lymphocytes of the BB dp rat. Binding is enhanced over that observed with lymphocytes from BB diabetic-resistant (dr) rats, which are able to generate CTL immune responses to LCMV and Pichinde viruses. Hence, lymphocytes from BB dp rats are uniquely susceptible to binding and replication of LCMV. During the acute state of LCMV infection, a general primary T-cell immunosuppression occurs with respect to a variety of viral and non-viral antigens. Over time, responsiveness to T-cell dependent antigens returns except for the ability to generate CTL responses to LCMV or the autoimmune response(s) required to cause insulin-dependent diabetes mellitus.

INTRODUCTION

BB diabetes-prone (dp) rats spontaneously develop insulin-dependent diabetes mellitus (IDDM), beginning at 60–150 days of age, stemming from specific, autoimmune destruction of beta cells of the islets of Langerhans' (Marliss *et al.*, 1982; Like *et al.*, 1982a; Nakhooda *et al.*, 1977; Dyrberg, Schwimmbeck & Oldstone, 1988; Schwimmbeck, Dyrberg & Oldstone, 1988; Oldstone *et al.*, 1990). At this time, inflammatory cells accumulate predominantly in the islets, and the disease can be passively transferred to the non-diabetic strain (BB dr) by concanavalin A(Con A)-stimulated lymphocytes from BB dp diabetic rats (Koevary *et al.*, 1983). Further, immunosuppression generated by such means as neonatal thymectomy or cyclosporin A treatment prevents the occurrence of IDDM in BB rats (Like *et al.*, 1982b; Laupacis *et al.*, 1983). We previously noted (Dyrberg *et al.*, 1988; Schwimmbeck *et al.*, 1988) that following inoculation of LCMV Armstrong 53b Clone 13 into BB dp rats, the expected incidence of IDDM declined significantly, blood glucose and pancreatic insulin levels became normal and lymphocytic infiltration in the islets of Langerhans' was minimum. Acute infection of lymphocytes and reduction of T lymphocyte subsets occurred transiently (Schwimmbeck *et al.*, 1988), but there was no evidence of persistent infection (Schwimmbeck *et al.*, 1988; Oldstone *et al.*, 1990). The virus, which was easily recoverable from lymphocytes by co-cultivation and from sera by plaquing during the first 15–20 days following infection, was no longer recoverable 30 days after infection nor through the next 220+ days of observation. In another species of acutely LCMV-infected mice, the virus similarly replicated in cells of the immune system (Buchmeier *et al.*, 1980; Doyle & Oldstone, 1978; Popescu, Löhler & Lehmann-Grube, 1979) and altered immune responsiveness to several antigens (Buchmeier *et al.*, 1980; Leist, Ruedi & Zinkernagel, 1988; Roost *et al.*, 1988).

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In this report, the overall immune responsiveness of BB dp rats acutely infected with LCMV was evaluated by using a battery of non-viral antigens. Immune responses were assessed to the T-cell dependent antigens bovine serum albumin (BSA), keyhole limpet haemocyanin (KLH), sheep red blood cells (SRBC) and human immunoglobulin G (HuIg). Additionally, the cytotoxic T lymphocyte (CTL) responses to LCMV and Pichinde virus and LCMV antibody response were examined. We found that (i) BB dp rats either uninfected or infected with LCMV do not generate a primary response to BSA; (ii) acute LCMV infection transiently suppresses immune responses to KLH, SRBC and HuIg; (iii) neither a primary nor a secondary LCMV CTL response develops; and (iv) LCMV binds preferentially to lymphocytes of the T-helper and cytotoxic/suppressor T subsets of BB dp compared to those of BB dr rats.

MATERIALS AND METHODS

Animals

The BB dp and dr rat sublines were obtained from the Worcester colony (Dr D. Juberski, University of Massachusetts, Worcester, MA) in approximately the 20th generation of inbreeding. The BB rats were propagated in the vivarium of the Research Institute of Scripps Clinic (RISC, La Jolla, CA) by continuous brother × sister matings. In the subsequent 5–8th generations bred at RISC, and used for the experiments here, the incidence of IDDM in BB dp rats varied from 74% to 85%, < 5% in BB dr rats.

Immunizations, antibody and CTL determinations

Rats were weaned and inoculated with LCMV at 30 days of age. One-half of the rats from each litter was given 1×10^7 plaque-forming units (PFU) of LCMV Armstrong (ARM) Clone 13 by intravenous injection into the tail vein. Seven days later, both LCMV-inoculated and uninfected rats were given either BSA, KLH, HuIg or SRBC. Groups of seven to 10 recipients of each antigen were bled at 7 and 28 days after the first inoculation. When rats were 63 days old (35 days after initiating primary immunization), they received a secondary inoculation of antigen. Blood was collected 7 days later; sera were then obtained and frozen at −20° until assayed. Antibody titres were determined in serial dilutions of sera using an ELISA (Gnann *et al.*, 1987). With high titres of known antibodies against BSA, KLH and HuIg (Oldstone *et al.*, 1973), the optimal dilution of antigen used to coat plates was determined and varied from 0·5 to 1 μg of antigen per well.

Two injections of 200 μg of BSA or KLH were injected subcutaneously (s.c.) into BB rats 4 weeks apart, the first inoculation occurring 7 days after rats received LCMV.

HuIg cohn fraction 2 was added to a DEAE–cellulose column equilibrated at 0·0175 M, pH 8·0, with potassium phosphate buffer. The eluent at a concentration of 10 mg/ml was immunochemically pure IgG by both immunoelectrophoresis and Ouchterlony analysis. Rats were injected s.c. with 0·2 ml of incomplete Freund's adjuvant containing 0·2 mg of HuIg on Day 0 and 28 days later.

To test for antibodies to SRBC, each rat was immunized with two intraperitoneal (i.p.) inoculations, 28 days apart, of 5×10^6 SRBC in a volume of 0·5 ml of 0·01 M sodium phosphate, 0·15 M NaCl, pH 7·2. Preliminary studies indicated that, of several doses utilized, this one led to an appropriate response in uninfected animals. Rats were bled by cardiac puncture 7 days after the primary and 7 days after the secondary inoculations. Haemagglutinins were titred in individual samples as described elsewhere (Oldstone *et al.*, 1973) utilizing two-fold dilutions of antiserum in phosphate-buffered saline (PBS) containing 1% heat-inactivated normal rat serum. Agglutination patterns were read after incubation at room temperature for 6 and 18 hr.

The cytotoxic activity of CTL was determined by a standard 5-hr ^{51}Cr-release assay (Whitton, Southern & Oldstone, 1988). BB dp, BB dr or MC57 target cells were infected with LCMV ARM Clone 13 or Pichinde virus at a multiplicity of infection (MOI) of 1, 48 hr prior to assay. To generate CTL, rats or mice were inoculated i.p. with 1×10^5 PFU of viruses. Six to 9 days after inoculation, spleens were harvested, and a single lymphocyte suspension free of red cells and macrophages made. These were added to target cells at effector : target ratios of 50 : 1, 25 : 1 and 12·5 : 1. The source, characterization and handling of the viruses used have been recorded elsewhere (Whitton *et al.*, 1988; Ahmed *et al.*, 1984). Secondary cytotoxic effector cells were generated by immunizing BB rats or C57BL/6 mice i.p. with 2×10^5 PFU of LCMV ARM. Six weeks later, their spleens were removed and restimulated *in vitro* with syngeneic LCMV ARM-infected peritoneal exudate macrophages. In brief, peritoneal exudate cells were induced by injecting 3 ml of 3% w/v thioglycolate (Difco, Detroit, MI) i.p. 3 days before harvesting. For restimulation, 2×10^5 macrophages, irradiated with 2000 rads, and infected 48 hr previously with LCMV ARM (MOI 3), were incubated with 4×10^6 spleen cells per ml of RPMI-1640 containing 10% fetal calf serum (FCS) 5×10^{-5} M 2-mercaptoethanol, penicillin and streptomycin. After 5 days of culture at 37°, effector cells were harvested and tested by the ^{51}Cr-release assay. Specific ^{51}Cr-release was calculated as

$$100 \times \frac{\text{c.p.m. sample} - \text{c.p.m. spontaneous release}}{\text{c.p.m. of total release} - \text{c.p.m. spontaneous release}}.$$

Neutralizing LCMV antibodies

Serum was obtained from blood collected by heart puncture from BB dp rats 4, 7, 10, 14, 30, 60, 90 and 180 days after LCMV inoculation. The presence of LCMV-specific neutralizing antibodies was assayed as described elsewhere (Schwimmbeck *et al.*, 1988). Briefly, five-fold dilutions of serum (heat-inactivated at 56° for 30 min) were incubated with a standard amount of LCMV Clone 13 at 37° for 45 min and then plated on a confluent layer of Vero cells. The end titre of neutralizing antibody reflected a 50% reduction in LCMV PFU.

Binding of LCMV to BB rat lymphocytes

LCMV stock was purified from BHK-infected cells as described elsewhere (Oldstone, 1990). Purified virus 1–2 mg protein/ml was incubated with N-hydroxysuccinimide-biotin diluted in dimethylsulphoxide at 1 mg/ml (Oldstone, 1990; Ingharimi *et al.*, 1988). A ratio of five parts virus to 1 part of biotin was used. After removing free biotin by dialysis against PBS, aliquots of biotinylated virus were diluted in RPMI medium containing 7% inactivated FCS and titred for infectious virus by plaquing. Biotinylated virus used in subsequent experiments showed a loss in infectivity of < 60% and was stored at −70° until used.

Before use, varying amounts of biotinylated virus (0·5–30 μg) were added to 1×10^6 lymphocytes and incubated on ice for 45 min. Thereafter, cells were washed twice, and avidin–

phycoerythin (PE) was added (Oldstone, 1990; Ingharimi *et al.*, 1988). After an additional 30 min incubation on ice, cells were washed and analysed on the fluorescence-activated cell sorter (FACS) to determine the amount of virus–biotin required for maximal binding to cells.

For experimental studies, monoclonal antibodies (50 λ) to various rat lymphocyte subsets and biotinylated-LCMV (50 λ) were added simultaneously to 1×10^6 lymphocytes. Cells were gently resuspended every 10 min and incubated for 45 min on ice. Cells were then washed twice in medium containing 5% heat-inactivated FCS. For double labelling, conjugated (fluorescein isothiocyanate; FITC) affinity-purified antibody to the immunoglobulin of the monoclonal antibody was added to mark the lymphocyte subset, while avidin–PE was added to mark the LCMV–biotin complex. Such treated cells were then gently suspended every 10 min during a 30-min incubation on ice. After three washes, the cells were treated with 0·5% formalin in PBS and analysed by FACS. Preliminary studies showed a dose–response curve for differing concentrations of LCMV–biotin and the blocking of LCMV–biotin binding by addition of unlabelled virus (A. Tishon and M. B. A. Oldstone, manuscript submitted for publication). These studies were performed on MC57 and BALB Clone 7 cells (Whitton *et al.*, 1988), to which binding was >75%. The source, dilution and usage of monoclonal antibodies to total rat T lymphocytes (W3/13), to rat CD4 T-helper lymphocytes (W3/25) and to non-helper CTL and natural killer (NK) cells (OX8) have been described elsewhere (Schwimmbeck *et al.*, 1988).

Replication of LCMV in BB rat lymphocytes

Replication of virus was determined by infective centre analysis (Schwimmbeck *et al.*, 1988; Doyle & Oldstone, 1978). Briefly, 7 or 21 days after 30-day-old BB rats received LCMV, lymphocytes were separated from their blood, purified and co-cultured with Vero cells. At least three rats from dp and dr groups were studied simultaneously per time-point. A lymphocyte able to replicate virus formed a plaque on the Vero cell. Three different concentrations of lymphocytes were added to a standard amount of Vero cells. The assay was run with duplicate samples and included positive (LCMV plaqued on Vero cells) and negative (neither virus nor lymphocytes) controls (Schwimmbeck *et al.*, 1988; Doyle & Oldstone, 1978).

RESULTS

BB dp rats fail to generate primary immune responses to BSA

BB dp rats, whether uninfected or acutely infected with LCMV, responded poorly when challenged with BSA. As shown in Fig. 1 neither group was able to mount a primary immune response. Secondary responses were weak but equivalent in LCMV-infected and uninfected control rats.

Immune responsiveness to KLH and SRBC

BB dp rats that were acutely infected with LCMV 7 days previously failed to generate primary immune responses to either KLH (Fig. 2) or SRBC (Fig. 3). However, upon rechallenge with KLH the secondary reponse to KLH was vigorous and equivalent in BB dp rats whether uninfected or acutely infected with LCMV (Fig. 2). In contrast, LCMV-infected rats receiving a secondary challenge of SRBC responded less well than their uninfected littermates (Fig. 3).

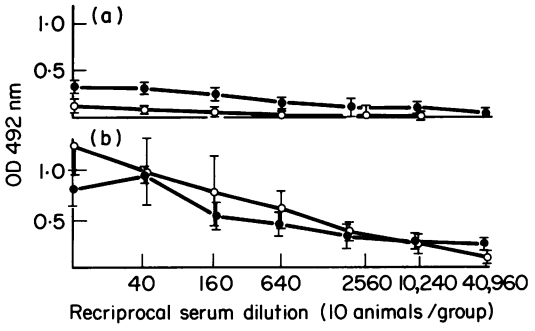

Figure 1. BB dp rats fail to mount primary humoral immune responses to BSA. Two-hundred micrograms of aggregated BSA were injected s.c. into 37-day-old BB dp rats, and their blood was assayed for the primary immune response 28 days later (a). After being bled, rats were reimmunized with 200 μg aggregated BSA and bled 7 days later to determine secondary response (b). The infected rats had been inoculated with LCMV (1×10^7 PFU of Clone 13 i.v.) 7 days preceding primary immunization with BSA. (●) LCMV infected; (○) uninfected.

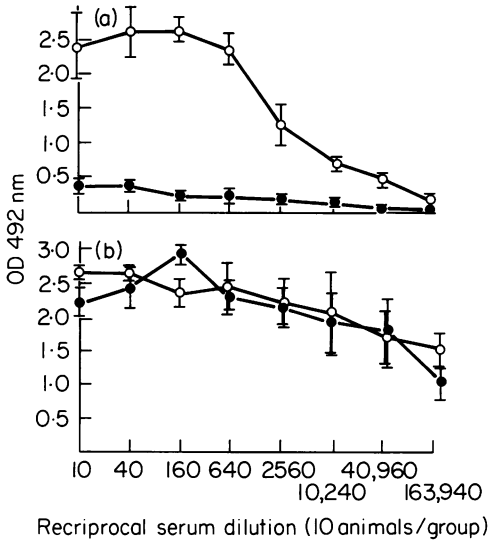

Figure 2. BB dp rats infected with LCMV fail to make a primary (a) but make a secondary (b) immune response to KLH. Two-hundred micrograms of KLH were injected s.c. into 37-day-old BB dp rats, whose blood was assayed for the primary immune response 28 days later. After the blood drawing, rats were reimmunized with 200 μg aggregated KLH and bled 7 days later to measure the secondary response. BB dp virus-infected rats were inoculated with LCMV (1×10^7 PFU of Clone 13 i.v.) 7 days prior to primary immunization. (●) LCMV infected; (○) uninfected.

Immune responsiveness to HuIg

LCMV-infected and uninfected BB dp rats mounted primary and secondary immune responses to HuIgG (Fig. 4). However, responses of the LCMV-infected animals were significantly poorer at both primary and secondary immune responses.

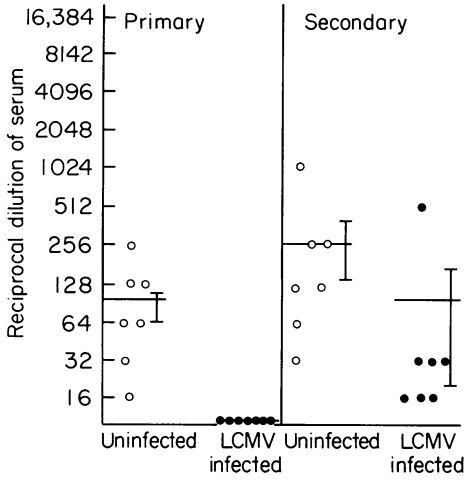

Figure 3. BB dp rats infected with LCMV fail to make a primary but mount a weak secondary response to SRBC. SRBC were diluted in PBS and 5×10^6 cells injected i.p. Sera was drawn 7 days later (primary response). Twenty-eight days after receiving the primary inoculation rats were reimmunized with 5×10^6 SRBC i.p., and blood was drawn 7 days thereafter (secondary response). BB dp virus-infected rats were inoculated with LCMV (1×10^7 PFU of Clone 13 i.v.) 7 days prior to primary immunization.

Primary and secondary CTL and antibody responses to LCMV

BB dp rats immunized i.p. with 1×10^5 PFU of LCMV failed to mount either a primary or secondary CTL response to LCMV (Fig. 5). Such rats also failed to generate CTL responses to Pichinde, a member of the arenavirus family that shows no cross-reaction at the CTL level. BB diabetes-resistant (dr) rats, concurrently inoculated with LCMV as a positive control, responded vigorously to both LCMV and Pichinde virus. Despite failing to generate CTL responses, BB dp rats developed high titres of neutralizing antibodies to LCMV, first appearing 10–15 days following infection and lasting over a 200-day observation period (data not shown; Schwimmbeck *et al.*, 1988).

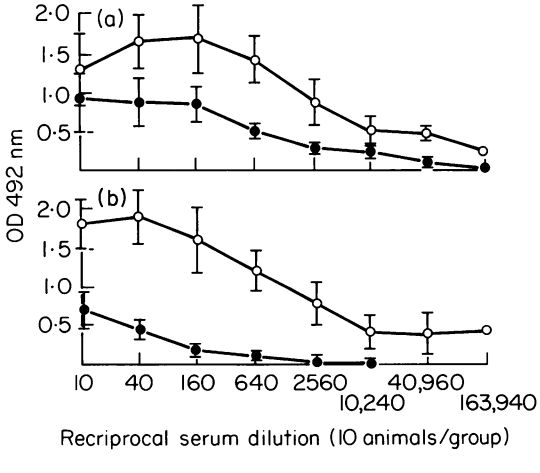

Figure 4. BB dp rats, infected with LCMV, mount poor primary (a) and secondary (b) responses to HuIg. 0·2 mg of IgG mixed with incomplete Freund's adjuvant was given s.c. to 37-day-old rats, and their blood was assayed for primary humoral response 28 days later. Immediately after bleeding, 0·2 mg of HuIg mixed with incomplete Freund's adjuvant was readministered, and rats were bled 7 days later. BB dp rats were inoculated with LCMV (1×10^7 PFU of Clone 13 i.v.) 7 days prior to primary immunization. (●) LCMV infected; (○) uninfected.

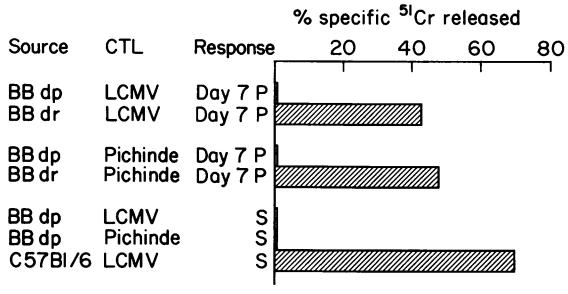

Figure 5. BB dp rats fail to form primary (P) or secondary (S) CTL to LCMV or Pichinde virus. Seven days after LCMV or Pichinde virus was given, 1×10^5 PFU i.p., to 30-day-old BB dp and BB dr rats, their spleens were removed and harvested lymphocytes reacted against ^{51}Cr-labelled BB dp targets. Results represent triplicate experiments. For the secondary CTL response, BB rats or C57BL/6 (H-2^b) mice were given LCMV or Pichinde virus (1×10^5 PFU). Sixty days later, their spleens were removed; the splenic lymphocytes were cultured for 5 days and then reacted against ^{51}Cr-labelled BB dp or MC57 (H-2^b) targets.

The last series of experiments evaluated the ability of LCMV to bind to and infect lymphocytes from BB dp and from BB dr rats. In the first set of studies, LCMV was conjugated to biotin and added to purified peripheral blood lymphocytes or to lymphocyte subsets defined by monoclonal antibodies and FITC-labelled reagents. Thereafter, the mixture was reacted with avidin–PE and subjected to FACS analysis. LCMV binding to total T cells (Fig. 6; W3/13 marked), T-helper (W3/25; data not shown) and cytotoxic T (OX8 labelled; data not shown) subsets from BB dp rats was greater than to corresponding lymphocyte subsets from BB dr rats. In multiple-repeat experiments, the enhancement of binding to BB dp over BB dr rat lymphocytes exceeded five-fold, ranging from five- to 12-fold.

Schwimmbeck, Dyrberg & Oldstone (1988) and others (Marliss *et al.*, 1982; Like *et al.*, 1982a; Nakhooda *et al.*, 1977) have noted a lymphocytopenia in BB dp compared to BB dr rats. During acute LCMV infection, BB dp rats had approximately 50% fewer lymphocytes of the W/13$^+$, W3/25$^+$ and OX8$^+$ subsets than uninfected or LCMV-infected BB dr rats (Schwimmbeck *et al.*, 1988; data not shown). By 14 days after infection, the lymphocyte levels in BB dp rats returned to normal.

The number of BB dp lymphocytes replicating virus was always significantly higher than the number of lymphocytes from BB dr rats. Hence in several experiments at 7 and 21 days after initiating acute LCMV infection, the number of infectious centres was (nine rats per group, mean $\pm$ 1 SD) 1423 ± 150 (range 1780–1200) and 459 ± 106 (range 730–110) per 1×10^5 BB dp lymphocytes, respectively. For BB dr rats at corresponding times, the numbers were 153 ± 59 (range 270–76) and 45 ± 12 (range 90–20) (Fig. 7).

DISCUSSION

These results demonstrate three major points. First, BB dp rats acutely infected with LCMV develop a generalized T-cell

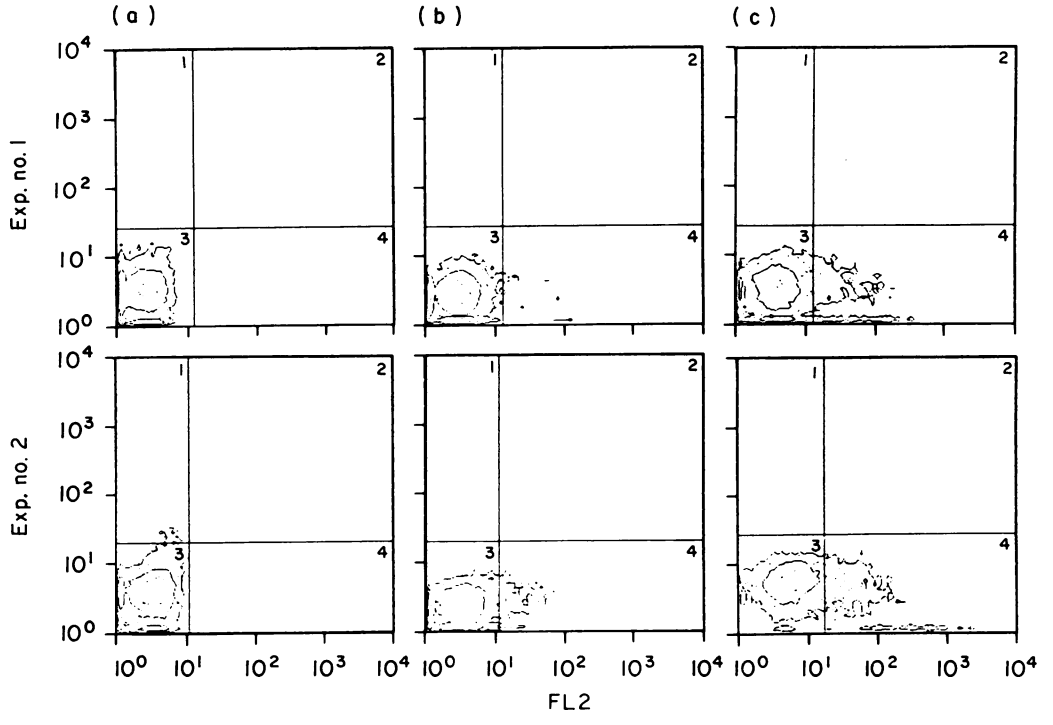

Figure 6. Biotinylated LCMV binds preferentially to lymphocytes from BB dp rats compared to those from BB dr rats. Ficoll–Hypaque-purified lymphocytes from BB dp and BB dr rats were incubated first with biotinylated LCMV and then avidin–PE. Cells were studied by the FACS. The two experiments shown were similar to three others. (a) BB dr negative control (no labelled virus); (b) BB dr plus biotinylated LCMV; (c) BB dp plus biotinylated LCMV.

immunosuppression so that no primary immune responses are generated to a variety of non-viral T-dependent antigens. However, by 28 days after injection of the infecting virus, the immune repertoire of BB dp rats is mostly functional, as evident when reinoculation of antigens leads to immune responses. Second, BB dp rats are apparently unable to generate CTL responses. Such rats fail to make either a primary or secondary CTL response to LCMV or a primary CTL response to Pichinde virus. In contrast, BB dr rats generate clear-cut virus-specific CTL responses. In addition, BSA is a poor immunogen for BB rats. Third, BB dp lymphocytes are more reactive with LCMV than lymphocytes from BB dr rats. Hence, both binding and replication of virus in lymphocytes from BB dp rats are enhanced.

LCMV clearly induces immunosuppression (Buchmeier *et al.*, 1980; Ahmed *et al.*, 1984; Woda & Padden, 1987; Lynch *et al.*, 1989; Leist *et al.*, Zinkernagel, 1988; Roost *et al.*, 1988; Wu-Hsieh, Howard & Ahmed, 1988; Oldstone *et al.*, 1988, 1986). In persistently infected mice, viral variants are generated in lymphocytes and these variants specifically abort LCMV CTL responses and are able to initiate persistence in adult immunocompetent mice (Ahmed *et al.*, 1984; Oldstone *et al.*, 1986). However, immune responses to several other viruses, including CTL responses, and immune responses to non-viral antigens are generally normal in such persistently infected mice (Buchmeier *et al.*, 1980; Oldstone *et al.*, 1973, 1986). In acutely LCMV-infected mice, lymphocytes sustain short-lived infection (Doyle & Oldstone, 1978) and an associated immunosuppression follows (reviewed by Buchmeier *et al.*, 1980; Lynch *et al.*, 1989; Leist *et al.*, 1988; Roost *et al.*, 1988; Wus-Hsieh *et al.*, 1988). Thus, in the mouse, LCMV is capable of causing transient and generalized immunosuppression during the acute phase of infection but, during persistent infection, a continuous and selective immunosuppression occurs. During persistent infection, suppression prevents the efficient generation of LCMV-specific CTL, thereby allowing the virus to survive. It was found here that, in the BB dp rat, LCMV causes an acute and transient suppression of several T-dependent immune responses, to KLH, Ig, SRBC, but not T-dependent antibody to LCMV.

The inability of BB dp rats to generate LCMV-specific CTL is consistent with an earlier report of Woda & Paddon (1987). These findings have been extended by showing that secondary CTL responses specific for LCMV are also absent. This inability to generate LCMV-specific CTL is probably a general defect of CTL induction, since it was found that BB dp rats generate no CTL to Pichinde (Fig. 5) or vaccinia viruses (Oldstone *et al.*, 1990), while others (Woda & Padden, 1987; Prud'homme *et al.*, 1988) have noted that allogeneic CTL cannot be elicited and that T cells from BB dp rats are unable to lyse their own islet cells *in vitro*. These data suggest two scenarios. First, CTL probably do not play a significant role in the autoimmune destruction of islet cells. Second, BB dp rats efficiently and effectively clear an acute virus infection despite the inability to generate CTL. As shown elsewhere, clearance and immunity are associated with the generation and maintenance of high-titred neutralizing antibody (Oldstone *et al.*, 1990).

During the first 10–15 days of acute LCMV infection, adult

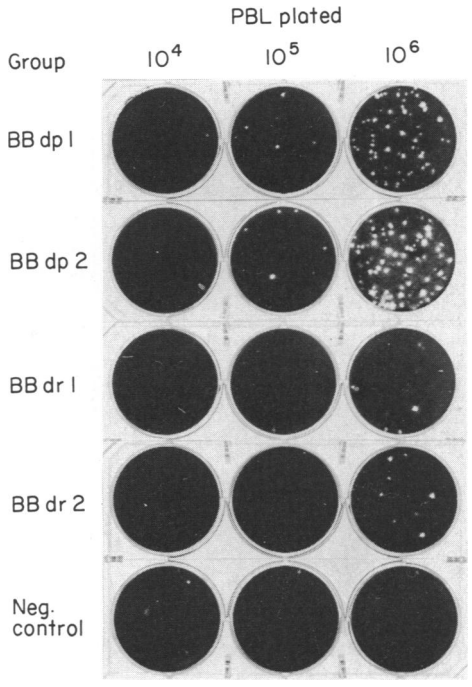

Figure 7. LCMV replicates preferentially in lymphocytes from BB dp rats compared to those from BB dr rats. Twenty-one days after LCMV was given (1×10^7 PFU i.v.) to 30-day-old BB dp and dr rats, lymphocytes were purified from their blood (Ficoll–Hypaque) and added in varying concentrations to a confluent monolayer of Vero cells. Six days later the material was stained (Schwimmbeck *et al.*, 1988; Doyle & Oldstone, 1978) and photographed. Approximately 10-fold more lymphocytes from BB dp rats score as infectious centres than from BB dr rats.

BB dp rats show virus-induced lymphocytopenia and accompanying immunosuppression. At this stage, the lymphocytes are permissive to viral invasion and replication. In the majority of BB dp rats by 21 days after infection, and in all by 30 days, lymphocyte levels have returned to normal (for BB dp) and virus is no longer recoverable from lymphocytes. In contrast, acutely LCMV-infected BB dr rats do not become lymphocytopenic (Schwimmbeck *et al.*, 1988) and do not exhibit a corresponding immunosuppression. Here, although LCMV bound to and replicated in lymphocytes from BB dp and BB dr rats, quantitatively the binding was at least five-fold enhanced for dp lymphocytes. Further, as judged by infectious centre analysis, five- to 10-fold more lymphocytes from BB dp rats replicated LCMV than those from BB dr rats. The enhanced replication of virus in BB dp rats correlated directly with their development of immunosuppression or a return to immunoresponsiveness. Interestingly, although these BB dp rats returned to a state of immune responsiveness against the T dependent non-viral antigens studied here, they nevertheless did not develop their expected IDDM over the next 300 days of observation (Dyrberg *et al.*, 1988; Schwimmbeck *et al.*, 1988). Thus, in some as yet undefined way, LCMV has suppressed the autoimmune IDDM function in an apparently permanent way while allowing a return to immune responsiveness. Further, no evidence exists that virus persists in such aged, IDDM-free BB dp rats (Schwimmbeck *et al.*, 1988; Oldstone *et al.*, 1990).

At least three applications of these results presented here seem evident. First, since LCMV infection of diabetic animals offers a number of similarities to the HIV–lymphocyte–AIDS picture (Leist *et al.*, 1988; Wu-Hsieh *et al.*, 1988; McChesney & Oldstone, 1989), the *in vivo* and *in vitro* models presented here suggest manipulative techniques for probing virus-induced immunosuppression. The restricted replication of LCMV to cells of the immune system and not to cells in other tissues (Schwimmbeck *et al.*, 1988) in adult BB dp hosts is especially interesting. Second, the differences noted in binding by and replication of virus in T cells of dp versus dr BB rats offer an opportunity to map the regulation of such responses. Third, eventually viruses, i.e. viral genes or their products, may be used to treat a spectrum of diseases. Like BB dp rats, NOD mice infected with a lymphotropic strain of LCMV do not develop IDDM (Oldstone, 1988), and lactate dehydrogenase virus prevents the spontaneous autoimmune disease, lupus, in New Zealand mice (Oldstone & Dixon, 1972) as well as experimentally induced allergic encephalomyelitis (Inada & Mims, 1986). These results *in toto* suggest that viruses can be used to manipulate immune responses in a beneficial way for the host and suggest the potential of therapeutic application.

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