VIRUSES AS THERAPEUTIC AGENTS

I. Treatment of Nonobese Insulin-dependent Diabetes Mice with Virus Prevents Insulin-dependent Diabetes Mellitus While Maintaining General Immune Competence

By MICHAEL B. A. OLDSTONE

From the Division of Virology, Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, California 92037

From their initial discovery (1–3), and to the present (4–6), viruses have been known as agents that cause disease. The site of injury depends upon the effect of viral genes or their expressed products on the cells or tissues infected, or upon the action of the immune response(s) that viruses induced (reviewed in references 7 and 8).

The immunologic system can be profoundly disordered through direct viral effects. Although such activity was observed clinically, over 150 yr ago (9), it fell to von Pirquet (10), later, to formally define direct interference by virus with the host's immune response. In that instance, measles virus infection caused a loss of the delayed hypersensitivity response to tuberculin antigen. This finding, along with the clinical observation that disorders like nephrotic nephrosis were relieved during acute measles virus infection (11, 12), led some to use this virus in the treatment of nephrosis (13) before the development of corticosteroids. These observations, the profound effect viruses have on cellular function coupled with their selective tropism, for particular cell types, led us to hypothesize that viruses, or probably their selected sequences or gene products, could be applied in therapeutic modalities and, hence, play a beneficial role in the treatment of human, domestic animal, and agricultural diseases (14, 15). Following this tack, we undertook experiments using a lymphotropic virus known to be relatively noncytolytic but to induce selective immunosuppression (reviewed in references 16–18) for treating autoimmune type 1 diabetes in nonobese insulin-dependent diabetes (NOD)[1] mice (14). Such mice spontaneously develop insulin-dependent diabetes mellitus (IDDM), frequently beginning by 6 mo of age with an incidence approaching 90% or better by the 9th to 12th month (14, 19–21). The diabetes has an autoimmune pathogenesis and is characterized by lymphocytic infiltration into the islet of Langerhans and β cell destruction (21–24). The end result is hypoinsulinemia, hyperglycemia, ketoacidosis, and death.

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Address correspondence to Michael B. A. Oldstone, Division of Virology, Department of Neuropharmacology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

[1] *Abbreviations used in this paper:* GP, glycoprotein; HuIg, human Ig; IDDM, insulin-dependent diabetes mellitus; KLH, keyhole limpet hemocyanin; NOD, nonobese insulin-dependent diabetes; NP, nucleoprotein; ORF, open reading frame; PFU, plaque-forming unit; S RNA, small segment RNA.

The agent chosen, lymphocytic choriomeningitis virus (LCMV), is a biosegmented RNA virus that is noncytolytic for the majority of cells it infects (16). Its inoculation into mice and rats leads to infection of lymphocytes primarily of the Th phenotype (25–27). Earlier, we showed that LCMV could prevent the occurrence of IDDM in NOD mice (14). In this paper, we analyze and extend this observation by demonstrating that: (*a*) the viral effect is on lymphocytes and not bone marrow cells; (*b*) the lymphocyte subset from NOD mice that is involved bears the Th-1.2$^+$ CD4$^+$ CD8^{nil} phenotype; and (*c*) several other CD4$^+$-dependent immune responses are not abrogated by LCMV infection, thus indicating that the effect on IDDM is selective and not generalized immunosuppression. In the accompanying paper, the component of the viral genome responsible for blocking IDDM is determined by use of genetic reassortants.

Materials and Methods

Mice. The NOD colony was established from breeder mice by brother-sister matings. Such breeders were obtained from K. Lafferty, University of Colorado Medical School, Denver, CO. The incidence of IDDM (defined as blood glucose of 300 mg/dl or over) was >90% in 9-mo-old mice with a mean accumulated blood glucose ± SEM of 463 ± 38 mg/dl.

Virus. The Armstrong strain of LCMV, clone 53b (ARM 53b), was used throughout these experiments. The origin, handling, and plaquing of this virus have been reported (17, 26). To establish persistent infection, newborn NOD mice <18 h of age were inoculated intracerebrally with 10^3 plaque-forming units (PFU) of ARM53b.

Biochemical and Molecular Analyses. Blood sugars were determined in blood serum or plasma using the glucose oxidase method, and pancreatic insulin was measured by RIA with rat insulin as a standard and A-14 ^{125}I-labeled bovine insulin as a tracer (14, 15). Insulin was extracted from the pancreas in acid ethanol, as described (28).

For in situ hybridization studies, lymphocytes from peripheral blood or spleens were purified by Ficoll-Hypaque gradient centrifugation, as described (26). Briefly, 10–15 ml of a 1:3 dilution of heparinized blood in PBS or a 10^8 single cell suspension from a spleen was placed in PBS, pH 7.2, and layered onto an equal volume of a mixture consisting of 12 parts of 14% (wt/vol) Ficoll (Pharmacia Fine Chemicals, Piscataway, NJ) and five parts of 32.8% (wt/vol) Hypaque (Winthrop Laboratories, New York, NY). The preparations were centrifuged at 600 *g* for 20 min at room temperature. Cells at the interface of the Ficoll-Hypaque and plasma were removed and washed in RPMI 1640 containing 10% heat-inactivated FCS. Residual contaminating erythrocytes were lysed with 0.83% ammonium chloride. The cells were washed again in MEM, and the number of viable lymphocytes per milliliter was determined with trypan blue.

Purified subsets of lymphocytes were obtained by using specific mAb, FITC fluorochrome dye, and FACS (26). Briefly, 10^6 lymphocytes were incubated in 100 μl supernatant (neat) from cells making mAb or 100 μl monoclonal ascites (1:50 dilution). mAbs used were 30.H12 anti-Th-1.2 supernatant, 53-6.72 anti-CD8$^+$ supernatant, and GK 1.5 anti-CD4 ascites. Lymphocytes were incubated on ice for 25 min, washed twice with RPMI 1640, and stained with affinity-purified F(ab')$_2$ fragment of mouse anti-rat IgG (H + L) FITC (5 μg/10^6 lymphocytes). After two washings with RPMI and one with PBS, lymphocytes were concentrated to ~4–6 × 10^6/ml PBS and sorted on the FACS. After establishing an initial cellular profile, the gate (window) was set to yield 99% or greater specific lymphocyte subset enrichment, and cells were resorted.

For in situ hybridization, lymphocytes obtained from Ficoll-Hypaque gradient centrifugation, or subsets obtained by FACS were concentrated to 10^7 cell/ml, and 5 μl were placed on each poly-L-lysine-coated glass slide. After air drying, the material was fixed in freshly made 2% paraformaldehyde-lysine-periodate, washed, treated with 0.2 M HCl, 1% Triton X-100, and 0.2% glycine in PBS, then dehydrated through graded ethanol washes. A hybridization mix containing the ^{35}S-LCMV riboprobe with formamide, dextran sulfate, sonicated

salmon sperm DNA, heparin dithiothretitol, Denhardt's solution, and appropriate salts was added as described (26, 29). After incubation, the slides were washed in SSC of various concentrations, dehydrated through graded ethanol washes, dipped in emulsion, placed in a darkened slide box, and left for a 3-d reaction at 4°C. Control consisted of hybridization of uninfected cell (negative control) and of LCMV-infected cells (positive control). These samples of uninfected and infected cells were placed on the same slide as the experimental sample and run through the procedure described above. In addition, an indifferent probe (^{35}S probe for CMV [29]) was reacted against materials harvested from LCMV-infected or LCMV-uninfected mice to ensure probe specificity.

The preparation and characterization of complementary DNA clones to both the large and small genomic RNA segments have been described (30, 31). Clones selected from the 5′ open reading frame (ORF) and the 3′ ORF of the small (S) RNA detect LCMV glycoprotein (GP) and nucleoprotein (NP), respectively (32). Clones from the 3′ end of the large RNA define the viral polymerase (large protein) (33). These clones have been labeled with ^{35}S by nick translation and used as hybridization probes. Briefly, LCMV cDNA inserts were excised from plasmid vectors and purified by gel electrophoresis before labeling. The average fragment length of the denatured probes was 200–400 bases, and the specific activity of ^{35}S-labeled DNA probe was 1–5 × 10^8 cpm/μg DNA.

Cell Transfers. The procedure followed was modified from that reported by Wicker et al. (22). Briefly, donors of splenic lymphocytes or bone marrow cells were LCMV-infected or -uninfected 7–9-mo-old female NOD mice. Recipients were 8–9-wk-old NOD female mice. Deletion of CD4, CD8, or Th-1.2 lymphocytes from the splenic population was achieved using mAbs and a complement source, as detailed elsewhere (25, 26). Each recipient received 5 × 10^7 splenic lymphocytes and 2 × 10^7 bone marrow cells (obtained from femurs). Before adoptive cell transfer, recipients received 850 rad from a ^{60}Co source. After transfer, mice were observed three times a week for weight loss, polydypsia, and polyuria, and were killed 30–40 d later.

Measurement of Immune Repertoire for Selected Th-dependent Antigens. Age- and sex-matched 6-wk-old untreated or LCMV persistently infected NOD mice were given either BSA, keyhole limpet hemocyanin (KLH), human IgG (HuIg), or SRBC. Individual groups of 10 mice given each antigen were bled 7 and 28 d after the first inoculation. 7 d later, they received a second inoculation of antigen. Blood was collected 7 d later, and sera were obtained and frozen at −20°C until assayed. Antibody determinations were done on serial dilution of sera using an ELISA (34). Using high titered antibodies against BSA, KLH, and HuIg (35), we determined the optimal dilution of antigen required to coat plates at 0.5–1 μg/ml.

BSA was introduced into mice subcutaneously. Two injections of 200 μg of BSA were given 4 wk apart. KLH was administered in a similar manner. HuIg, Cohn fraction II, 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, and mice were injected subcutaneously with 0.2 ml of IFA containing 0.2 mg of HuIg on day 0 and 28 d later. NOD mice were immunized with two intraperitoneal inoculations 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, each given 28 d apart. Preliminary studies indicate that, of several doses utilized, this one led to an appropriate response in uninfected animals. Mice were bled 7 d after the primary and 7 d after the secondary inoculation. Hemagglutinins were titered on individual samples as described (35).

Histology. Tissues were fixed in formalin and stained with hemotoxylin and eosin. Sections of pancreas were made 1/3, 1/2, and 2/3 into the tissue to obtain a sampling of the islets of Langerhans. In most instances, their histopathologic pattern was determined by counting at least 15 islets per pancreas.

Results

Persistent LCMV Infection of NOD Mice Aborts their IDDM. After inoculation with LCMV at birth, NOD mice became persistently infected. All mice carried the virus; i.e., 12 infected NOD mice randomly selected at 6 wk and 6 mo of age and tested

by plaque assay, contained a range of infectious virus from 10^4 to 6×10^5, with a mean ± 1 SD of 1.8×10^5 PFU/ml of sera. As shown in Fig. 1, such LCMV persistently infected NOD mice maintained normal blood glucose and pancreatic insulin titers over the next 11 mo. In contrast, and as expected, uninfected NOD litter mates progressively developed IDDM (Fig. 1). Hence, by 6 mo of age, 60% of such mice had blood sugar levels >300 mg/dl, and by 9 mo and thereafter, >95% had IDDM. While LCMV-infected NOD mice maintained normal levels of insulin in their pancreases, as determined by RIA (Fig. 1) and immunofluorescent staining of islets with antibody to insulin (data not shown), levels of pancreatic insulin in untreated NOD mice fell dramatically by 6 mo of age and thereafter. Their low pancreatic insulin levels inversely paralleled rising quantities of glucose in their blood.

The untreated, IDDM-afflicted NOD mice showed evidence of lymphocytic infiltration in the islets of Langerhans (Fig. 2 *1*) (19–21), whereas their LCMV-infected counterparts had little or no lymphocytic infiltration. Sectioning of islets at different locations throughout the pancreas showed that >75% of islets from LCMV-infected 9–12-mo-old NOD mice were free from autoimmune destruction.

LCMV Infection of CD4⁺ Splenic Lymphocytes Is Associated with Inhibition of IDDM. Since IDDM can be adoptively transferred (22), peripheral blood or splenic lymphocytes obtained from 7-mo or older NOD mice were transferred along with bone marrow cells into irradiated 8–9-wk-old NOD mice. As a result, the recipients developed IDDM within 4 wk (Table I). However, similar transfers of lymphocytes and bone marrow cells from the similarly aged LCMV-infected NOD donors into matched, young recipients did not cause IDDM (Table I). Further, the data in Table I and Fig. 2 indicate that LCMV infection of lymphocytes, not bone marrow cells,

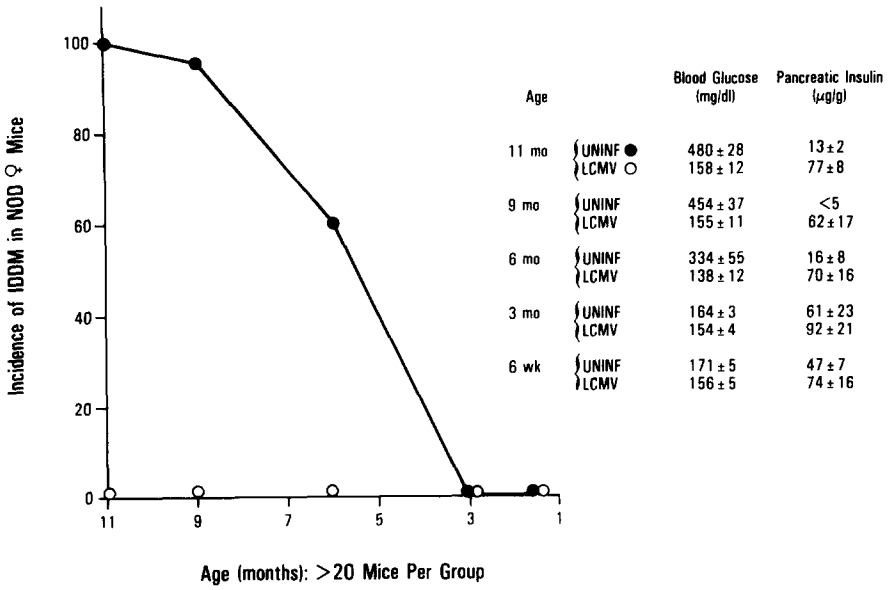


FIGURE 1. Occurrence of IDDM in uninfected (●) and LCMV-infected (○) NOD female mice. Over 20 mice per group were studied for blood glucose at each time point. Four to five mice were studied for levels of pancreatic insulin at 6 wk 3–9 mo, and 10 mice per group at 11–12 mo.

TABLE I

$CD4^+$ Lymphocytes Are Responsible for Transfer/Suppression of IDDM to Young NOD Female Recipients

Exp.	7-9-mo-old NOD female donors provide:		12-wk-old NOD female (uninfected) recipients 4 wk after adoptive transfer			
	Splenic lymphocytes	Bone marrow cells	Glucose value	GTT	Pancreatic insulin	Normal islets
				*mg/dl**	*μg/g*	%
1	Uninfected	Uninfected	358 ± 30	ND	0.8‡	<5§
	LCMV infected	LCMV infected	155 ± 9	ND	54.0	>75
	LCMV infected	Uninfected	167 ± 10	ND	42.0	>75
	Uninfected	LCMV infected	347 ± 63	ND	0.6	< 5
2	LCMV $CD4^+$ $CD8^+$	Uninfected	165 ± 10	230 ± 30 (1/5)	58	>75
	LCMV $CD4^{nil}$ $CD8^+$	Uninfected	292 ± 83	460 ± 14 (5/5)	18	<10
	LCMV $CD4^+$ $CD8^{nil}$	Uninfected	120 ± 7	181 ± 14 (0/5)	46	>75
	Uninfected	Uninfected	327 ± 42	ND	<1	<5

5×10^7 splenic lymphocytes and 2×10^7 bone marrow cells from femurs were transferred intravenously into 8-wk-old NOD mice. Before transfer, the recipients received 850 rad from a ^{60}Co source. Repeated experiments gave similar results.

* Four to five mice were in each group. The mean blood glucose ± 1 SE of recipients before adoptive transfer was 145 (range, 130–168) ± 8. GTT; glucose tolerance test. Mice were given 2 mg of glucose intraperitoneally per kg of body weight and bled 1 h later. Abnormal GTT consisted of a 2.5-fold or greater enhancement in elevation of glucose (see reference 47). The number represents mean glucose ± 1 SE, five mice per group. In parentheses are the number of mice with an abnormal GTT over the total mice per group.

‡ Micrograms of insulin per gram of pancreas, as determined by RIA (see Methods and Materials).

§ Percent of islets of Langerhans showing normal histology. A minimum of 15 islets studied per mouse.

prevented the IDDM. Thus, recipients receiving lymphocytes from LCMV-infected donors, but bone marrow cells from uninfected NODs, had normal blood glucose and pancreatic insulin levels and no mononuclear infiltration into the islets of Langerhans (Fig. 2 *3*). In contrast, recipients obtaining lymphocytes from uninfected NOD donors along with bone marrow cells from LCMV-infected NOD donors developed IDDM (Fig. 2 *2*; Table I).

Having identified splenic lymphocytes as the primary source for transfer/suppression of IDDM, we next sought the phenotype of the effector lymphocyte subset. Preliminary studies indicated that the cell was Th-1.2^+ when transfer of such cells from LCMV-infected mice into uninfected NOD recipients aborted the expected occurrence of IDDM. To further type the lymphocyte subset involved, either $CD4^+$ or $CD8^+$ cells were deleted from the LCMV splenic lymphocyte population used for adoptive transfer. According to concurrent FACS analysis of these populations, the deletion procedure always yielded at least 97% purity and was usually 99% efficient. As demonstrated in Table I and Fig. 2, *4* and *5*, when $CD4^+$ cells were deleted, IDDM occurred; in contrast, when $CD4^+$ cells from LCMV-infected NOD mice were transferred, the IDDM was suppressed. Further, when $CD4^+$ cells were deleted from the LCMV spleen population, the remaining $CD8^+$ cells in combination with bone marrow cells from uninfected mice were able to induce IDDM in young NOD recipients (Table I).

None of the recipient mice receiving LCMV-infected cells showed evidence of

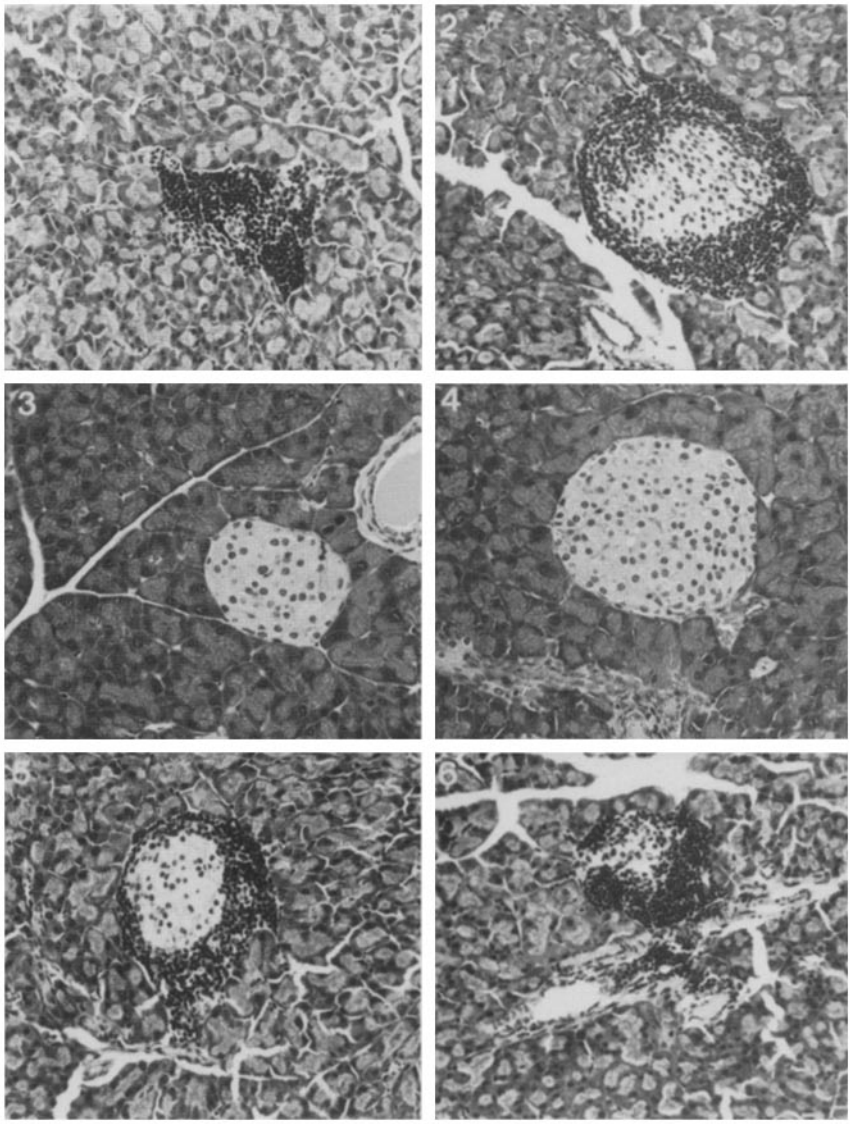
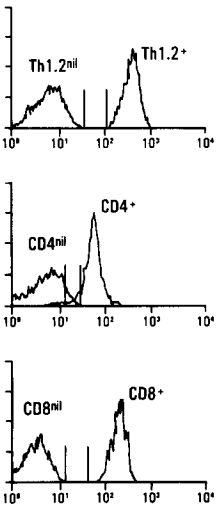


FIGURE 2. Results of adoptive transfer experiments using splenic lymphocytes from untreated and LCMV-infected 7–9-mo-old NOD female donors into 8-wk-old NOD female recipients. Photomicrographs show islets of Langerhans from (*1*) untreated 9-mo-old donor; (*2*) recipient of $CD4^+$ and $CD8^+$ lymphocytes from an uninfected mouse and bone marrow cells from an LCMV-infected mouse; (*3*) recipient of $CD4^+$ and $CD8^+$ lymphocytes from an LCMV-infected mouse and bone marrow cells from an uninfected mouse; (*4*) recipient of $CD4^+$ $CD8^{nil}$ lymphocytes and bone marrow cells from an LCMV-infected mouse; (*5*) recipient of $CD4^+$ $CD8^{nil}$ lymphocytes from an uninfected mouse and bone marrow cells from an LCMV-infected mouse; (*6*) recipient of lymphocytes and bone marrow cells from an uninfected mouse. (×425).

infectious LCMV in their blood at the time of death, indicating that replicating virus (sensitivity of assay, <50 PFU) was not released from transferred cells.

CD4⁺ Lymphocytes Preferentially Express LCMV Nucleic Acid Sequences. Next, we determined the phenotype of lymphocytes in peripheral blood and spleens of infected NOD mice that expressed LCMV nucleic acid sequences. Using in situ hybridization with ^{35}S LCMV cDNA probes, we found both LCMV GP and NP nucleic acid sequences present in lymphocytes from LCMV-infected mice of all ages. We then determined the lymphocyte subset(s) carrying viral sequences. The use of mAbs, double cell sorting, and FACS yielded population of lymphocytes with purity >99% (Fig. 3). When such purified populations were analyzed by in situ hybridization with a cDNA probe from the 5′ end of the small RNA, which encompassed most of the LCMV GP, LCMV nucleic acid sequences were found to be primarily sequestered in the Th-1.2⁺ CD4⁺ CD8^{nil} population (Fig. 3). Results were similar in splenic lymphocytes and their subsets, and with using cDNA probe to the virus NP or L regions.

LCMV Infection of a Subset of CD4⁺ Lymphocytes Does not Interfere with Generation of Immune Responses to Several T-dependent Antigens. The last series of experiments evaluated the ability of NOD mice, persistently infected with LCMV, to generate primary and secondary immune responses to SRBC (Fig. 4), BSA (Fig. 5), KLH (Fig. 6), and HuIg (Fig. 7). As seen in Figs. 4–7, except for their failure to generate a good secondary immune response to HuIg, 3-mo-old female NOD mice persistently infected with LCMV made efficient primary and secondary immune responses to all the antigens administered. Primary responses to HuIg (Fig. 7), and primary and secondary immune responses to BSA (Fig. 5), were equivalent in untreated and LCMV-infected NOD mice. Although LCMV-infected NOD mice generated good



LCMV NUCLEIC ACID SEQUENCES IN PERIPHERAL BLOOD LYMPHOCYTES

	#Cells Positive	Total #Cells	% +
Th1.2^{nil}	1	1950	0.05
Th1.2⁺	16	1150	1.39
CD4^{nil}	2	2400	0.08
CD4⁺	15	1250	1.20
CD8^{nil}	22	1210	1.81
CD8⁺	1	1500	0.07
PBL⁺ CONT	44	1260	3.49
UNINF CONT	18	17,100	0.11

FIGURE 3. Profile of Th-1.2^{nil}, Th-1.2⁺, CD4^{nil}, CD4⁺, CD8^{nil}, and CD8⁺ subsets harvested from PBL containing LCMV nucleic acid sequences. NOD mice were infected at birth with LCMV ARM 53b. When 3 mo old, 8–12 females were killed, their blood was pooled, and PBL were separated by Ficoll-Hypaque centrifugation and divided into fractions of 1–2 × 10^6 cells. Selected mAbs and a fluorochrome marker were used to identify various lymphocyte subsets that were segregated by two cycles of sorting by FACS. After the first cycle, windows on the sorter were set to ensure >99% enrichment of various lymphocyte subsets after the second FACS run. Subsets obtained were analyzed for LCMV sequences using a ^{35}S LCMV cDNA probe of the GP region. See Materials and Methods for details. At least 1,100 cells were counted and the percent positive (% +) was recorded. Data represent one of three experiments. Positive and negative controls were run concurrently and consisted of PBL from LCMV persistently infected and uninfected BALB mice. Corresponding results were obtained with PBL from 6-mo-old infected mice.

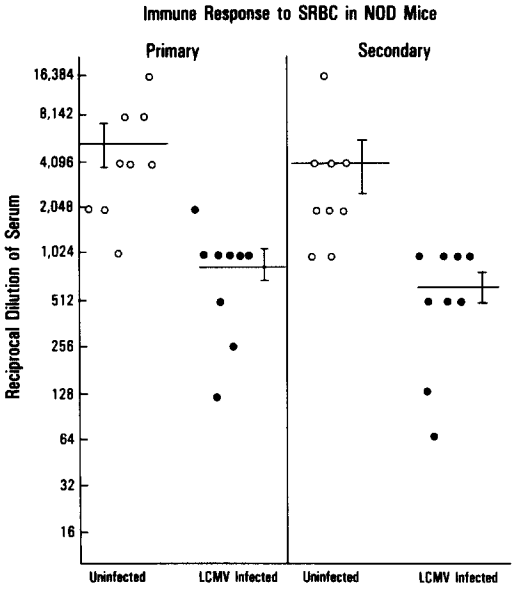


FIGURE 4. LCMV-infected 3-mo-old NOD mice (●) make primary and secondary immune responses to SRBC. Bar represents 1 SD above or below the mean value for 10 mice per group. (○) Age- and sex-matched uninfected mice.

primary and secondary immune responses to SRBC (Fig. 4), and secondary response to KLH (Fig. 5), these responses were, in general, less than those observed in uninfected NOD mice.

Discussion

Our studies clearly indicate that LCMV prevents the IDDM of NOD mice. These adult mice infected with LCMV in infancy maintain normal blood glucose levels

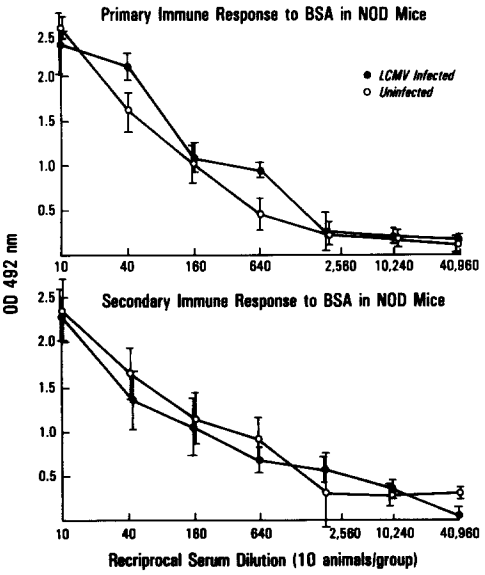


FIGURE 5. LCMV-infected 3-mo-old NOD mice (●) make primary and secondary immune responses to BSA that are equivalent to responses made in age- and sex-matched untreated mice (○). Bar represents 1 SD above and below the mean value for 10 mice per group.

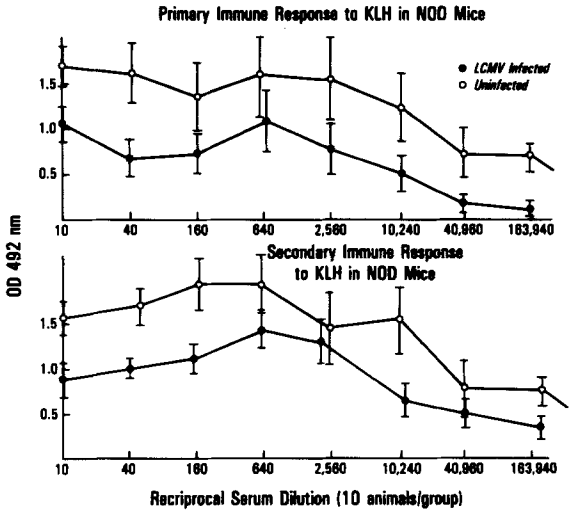


FIGURE 6. LCMV-infected 3-mo-old NOD mice (●) made primary and secondary responses to KLH that were lower but not significantly different from those made by age- and sex-matched untreated mice (○). Bar represents 1 SD above and below the mean value for 10 mice per group.

and biochemical integrity of their islets of Langerhans. Thus, the autoimmune destruction of islets typical for this strain is retarded or aborted, and their pancreatic insulin levels remain normal through 1 yr of age. In contrast, NOD mice not infected with LCMV, upon reaching their ninth month, uniformly have severe IDDM with hyperglycemia, lymphocytic infiltration into the islets that destroys them, and low pancreatic insulin levels.

The virus may abrogate IDDM through its action on a subset Th-1.2$^+$ CD4$^+$ lymphocytes. Lymphocytes from LCMV-infected NOD mice that bear the Th-1.2$^+$ CD4$^+$ CD8^{nil} phenotype fail to transfer IDDM, whereas similar cells from NOD mice not infected with LCMV do so. Bone marrow cells from uninfected NOD mice, when adoptively transferred with lymphocytes from LCMV-infected mice, do not transfer the disease, and bone marrow cells from the infected mice do not suppress IDDM when transferred with lymphocytes from the uninfected mice. These results

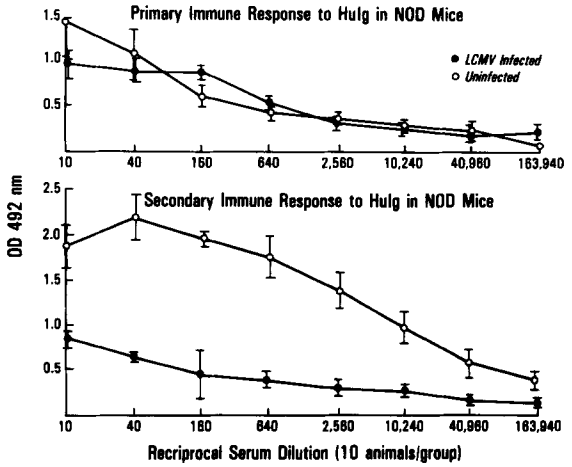


FIGURE 7. LCMV-infected 3-mo-old NOD mice (●) make primary immune responses to Hulg that is equivalent to the response made by age- and sex-matched untreated mice (○). However, those LCMV-infected mice fail to generate a good secondary response to Hulg ($p > 0.001$) when compared with uninfected mice. Bar represents 1 SD above and below the mean value for 10 mice per group.

are consistent with the report of Shizuru et al. (36), whose use of mAbs against the CD4 determinant prevented lymphocytic infiltration, eliminated destruction of insulin-producing β cells, and blocked IDDM in NOD mice (36). Similarly, several investigators have shown a selective role for $CD4^+$ lymphocytes in the ability to transfer IDDM in NOD mice (37–39), while others noted a combined or accelerated role with $CD4^+$ and $CD8^+$ lymphocytes (22, 40, 41). Recently, Reich et al. (42) suggested that $CD4^+$ lymphocytes bearing Vβ5 TCR were implicated in causing IDDM. Our preliminary experiments (A. Tishon and M. B. A. Oldstone, unpublished results), comparing numbers of Vβ5 bearing $CD4^+$ and $CD8^+$ lymphocytes in spleens and peripheral blood from four unmanipulated NODs with four LCMV persistently infected NODs, have yet to demonstrate differences between both groups.

The involvement of $CD4^+$ cells in offsetting this genetic susceptibility to disease of LCMV-infected NOD mice is not associated with generalized immunosuppression. Despite carrying LCMV and escaping from IDDM, these mice are nevertheless able to make immune responses to a wide variety of antigens all dependent on CD4 function. Thus, the virus is causing, in some yet to be explained manner, a selective and lasting immunosuppression against autoimmune diabetes without total suppression of immune responsiveness. The selective suppression of $CD4^+$ cells observed during virus infection is consistent with data showing that the cellular tropism of virus is restricted primarily to a small subset of $CD4^+$ lymphocytes. Thus, LCMV resides in Th-1.2$^+$ $CD4^+$ $CD8^{nil}$ peripheral blood and splenic lymphocytes during the span of the animals' lives, but in only ~1–2% of such $CD4^+$ cells. While the sensitivity of the in situ hybridization assay with LCMV, like studies with other viruses, detects ~50 LCMV gene copies per cell (26), the lack of a global suppression in Th-dependent immune responses argues against an overwhelming number of $CD4^+$ cells being involved. Thus, it is of interest to explore the similarities and differences between LCMV and HIV, as regards infection of a subset of $CD4^+$ lymphocytes and the associated selective (LCMV) or generalized (HIV) resultant immunosuppression. The fact that infectious LCMV is not recovered from the blood of recipient mice receiving $CD4^+$ LCMV-infected cells during adoptive transfer indicates that virus is likely not being released and transferred to uninfected lymphocytes in vivo. A similar conclusion was noted earlier (43) upon transfer of LCMV-infected lymphocytes from a parental donor into its uninfected F_1 offspring. Over a 3-mo study, only lymphocytes (donor phenotype), never blood, nor recipient's lymphocytes, contained LCMV materials (43).

LCMV is selective in aborting the autoimmune disease IDDM and does not suppress autoimmune diseases in general. For example, persistent LCMV infection of New Zealand mice enhances autoimmune responses and resultant disease in (NZB × NZW)F_1 and in NZB mice, as well as initiating disease in NZWs (44, 45). Therefore, study of LCMV infection offers two interesting areas for future exploration. First, it serves as a probe to assess and dissect regulation of the autoimmune response and the autoimmune basis for a number of autoimmune diseases. Second, because of selective immunosuppression, the virus, or better, one of its genes or products, can be useful as a therapeutic product. Hence, LCMV in the NOD mouse provides a model for experimental manipulation of a virus as a potential therapeutic agent in the treatment of disease. This latter issue is further addressed in the accompanying paper (46). There, we show that the whole viral genome is not needed to

abort IDDM of NOD mice. Rather, the suppressive effect of the virus resides in genes encoded on the smaller of the two LCMV RNA segments.

Summary

A situation in which virus can be used as a therapeutic agent to prevent a lethal autoimmune disease is explored. Nonobese insulin-dependent diabetes (NOD) mice spontaneously develop insulin-dependent diabetes mellitus (IDDM), characterized by lymphocytic infiltration into the islets of Langerhans and β cell destruction, resulting in hypoinsulinemia, hyperglycemia, ketoacidosis, and death. Infection of NOD mice with lymphocytic choriomeningitis virus (LCMV) aborts the autoimmune manifestations and resultant IDDM. The viruses' effect is on a subset of $CD4^+$ lymphocytes. Ablating this autoimmune diabetes does not significantly alter immune responses to a variety of non-LCMV antigens that require $CD4^+$ lymphocyte participation. The prevention of IDDM associated with viral therapy is maintained throughout the life spans of NOD mice.

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