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20. Abbreviations for the amino acid residues are: C, Cys; D, Asp; E, Glu; F, Phe; L, Leu; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; and V, Val.
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# Prevention of Type I Diabetes in Nonobese Diabetic Mice by Virus Infection

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**The nonobese diabetic (NOD) mouse is an animal model of type I diabetes and develops a characteristic autoimmune lesion in the islets of Langerhans with lymphocytic infiltration and destruction of pancreatic β cells. The result is hypoinsulinemia, hyperglycemia, ketoacidosis, and death. Diabetes usually begins by the sixth month of age but can occur earlier when young NOD mice are infused with lymphocytes from older NOD donors. When newborn or adult NOD mice were infected with a lymphotropic virus they did not become diabetic. The interaction between viruses and lymphocytes is pivotal in aborting diabetes, as established by experiments in which lymphocytes from virus-infected donors failed to transfer diabetes. In contrast, lymphocytes from age- and sex-matched uninfected donors caused disease. Therefore, viruses and, presumably, their products can be developed to be beneficial and may have potential as a component for treatment of human diseases. Further, these results point to the utility of viruses as probes for dissecting the pathogenesis of a nonviral disease.**

VIRUSES ARE KNOWN BY THE CELLS they injure or diseases they cause. According to P. and J. Medawar (*1*), "viruses make themselves known only by causing disease or other pathological changes; the existence of benign viruses having no ill effects remains conjectural. No virus is known to do good. It has been well said that a virus is a piece of bad news wrapped up in protein."

Certain viruses infect and replicate in lymphocytes, thereby disordering their function and causing immune suppression or heightened autoimmune responses (*2, 3*). Perhaps viruses could be directed to induce selective immune suppression in an autoimmune disorder, with the potential to prevent the autoimmune response and concomitant disease. This hypothesis was tested in a study of the nonobese diabetic (NOD) mouse, which spontaneously develops insulin-dependent diabetes mellitus (IDDM), usually beginning by 6 months of age, with an incidence of nearly 100% by the 9th to 12th month. The diabetes is autoimmune and involves lymphocytic infiltration around and into the islets of Langerhans with pancreatic β cell destruction. The result is hypoinsulinemia, hyperglycemia, ketoacidosis, and death, as in human IDDM (*4–6*).

Lymphocytic choriomeningitis virus (LCMV) is an ambisense bisegmented RNA virus that is a natural pathogen of mice (*7–9*). In most murine strains, injection at birth (immunoincompetent host) or in adulthood (immunocompetent host) with a lymphotropic variant of LCMV results in infection of lymphocytes, primarily of the T-helper subset (Thy $1.2^+$, $L3T4^+$, Lyt-$2^-$) (*10, 11*). The outcome is abrogation of virus-specific H-2–restricted cytotoxic T lymphocyte function, failure to clear virus, and viral persistence throughout the animal's lifespan (*9, 12*). LCMV infection initiated in NOD mice at birth or in adulthood abrogated the expected incidence of IDDM and normalized blood glucose and pancreatic insulin levels. Furthermore, adoptive transfer of lymphocytes from LCMV-infected NOD mice into uninfected NOD recipients prevented or minimized the autoimmune lesions within islets; in contrast, NOD mice littermates that received lymphocytes from uninfected mice had clear-cut IDDM.

The NOD colony was established from breeder mice by brother-sister matings. The incidence of IDDM (defined as blood glucose greater than 300 mg/dl) in twenty 9-month-old mice was 95%, with the mean ± SEM blood glucose of 454 ± 37 mg/dl.

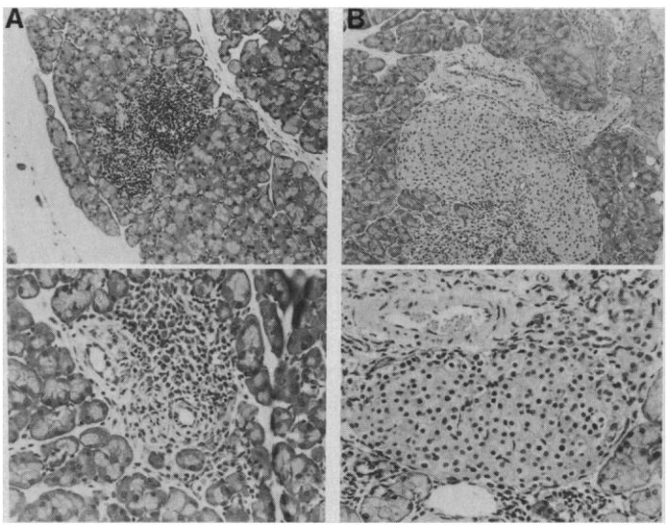


**Fig. 1.** Histopathology of pancreatic islets of NOD mice 30 days after exposure to radiation (850 rads) and subsequent adoptive transfer of lymphocytes and bone marrow cells from (**A**) an uninfected NOD mouse and (**B**) a NOD mouse infected at birth with LCMV. Massive islet cell destruction and lymphocytic infiltration can be observed in two separate mice receiving lymphocytes from the uninfected donor (A) as contrasted to two mice receiving lymphocytes from virus-infected donors and failing to develop IDDM. Magnifications in (A) and (B): top, ×250; bottom, ×450. These results are representative of individual mice in the experimental groups recorded in Table 2. See Table 2 for experimental details.

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However, when newborn NOD mice were injected intracerebrally with $10^3$ plaque-forming units (PFU) of the Armstrong strain of LCMV (ARM53b), or when 6-week-old NOD adults received intravenously $1 \times 10^6$ PFU of LCMV ARM53b clone 13, a lymphotropic variant, the incidence of IDDM decreased to 0% and 6%, respectively, during the same interval. Similarly, the average value for blood sugar was 155 ± 11 mg/dl in mice infected at birth and 206 ± 16 mg/dl in mice infected as adults (Table 1). Thus, virus infection of both newborn and adult NOD mice successfully aborted the expected incidence of IDDM.

IDDM can be adoptively transferred by lymphocytes in the NOD mouse model (*13*). Peripheral blood lymphocytes obtained from eight NOD mice infected with LCMV contained viral nucleic acid sequences [from 0.5 to 7% (average 2%) of lymphocytes harbored viral sequences]. Furthermore, when 4-month-old uninfected NOD mice (preceding the onset of IDDM) (Table 1) were irradiated (850 rads) from a cobalt-60 source and were then reconstituted intravenously with $2 \times 10^7$ bone marrow cells and $5 \times 10^7$ splenic lymphocytes from 6-month-old uninfected NOD mice, IDDM developed in 80% (four of five) within 30 days (Table 2). In contrast, splenic lymphocytes and bone marrow cells from LCMV-infected mice of the same age prevented IDDM in all the infused recipients (Table 2). Lymphocytes from donors with persistent infections fail to release infectious virus and thus do not infect recipient mice, as shown elsewhere by adoptive transfer of lymphocytes from LCMV-infected $F_1$ recipients into uninfected parental strains (*14*) and here by the failure to detect infectious virus in the sera of recipients (sera of donors contained $1 \times 10^4$ to $5 \times 10^5$ PFU per milliliter of serum, whereas none of the five recipients had any detectable virus in their sera). Infectious virus can be recovered from these lymphocytes, but only when they are cocultured on a substrate of susceptible Vero cells (*10, 11, 14, 15*). These observations, coupled with evidence of viral sequences in transferred lymphocytes, indicate that the prevention of IDDM is most likely caused by virus-induced inactivation of potentially autoimmune reactive lymphocytes, not by introduction of infectious virus into recipient mice.

**Table 1.** Virus infection prevents diabetes in NOD mice. Female NOD mice were used and injected intracerebrally with $10^3$ PFU of LCMV ARM53b at birth, or intravenously with $1 \times 10^6$ PFU of LCMV ARM53b clone 13 at 6 weeks of age. Results with ARM53b or ARM53b clone 13 given to newborns were equivalent. Stock of virus used, its passage history, monitoring of virus infection, and quantitation of PFU have been reported (*22, 23*). Blood sugar was determined by the glucose oxidase method. Incidence of IDDM is reported as the number of NOD mice having blood glucose >300 mg/dl over the total number of mice per group. Blood glucose is reported as the mean ± SEM for all mice in the group.

| Age of mouse | Characteristic | IDDM (blood sugar >300 ng/dl) | |
|---|---|---|---|
| | | Incidence | Blood glucose (mg/dl) |
| 6 weeks | Uninfected | 0/20 | 171 ± 5 |
| | Virus at birth | 0/19 | 156 ± 2 |
| | Virus at 6 weeks | 0/19 | 165 ± 5 |
| 3 months | Uninfected | 0/21 | 164 ± 3 |
| | Virus at birth | 0/19 | 154 ± 4 |
| | Virus at 6 weeks | 0/18 | 162 ± 4 |
| 6 months | Uninfected | 12/20 | 334 ± 35* |
| | Virus at birth | 0/19 | 126 ± 6 |
| | Virus at 6 weeks | 0/18 | 190 ± 20 |
| 9 months | Uninfected | 19/20 | 454 ± 37* |
| | Virus at birth | 0/19 | 155 ± 11 |
| | Virus at 6 weeks | 1/17 | 206 ± 16 |

*$P < 0.001$ compared to either virus at birth or virus at 6 weeks.

**Table 2.** Prevention of diabetes in NOD mice with transfers of lymphocytes from virus-infected donors. Donors were 6- to 7-month-old uninfected female NOD mice or 8- to 10-month-old female NOD mice infected at birth with LCMV ARM53b. Bone marrow cells from femurs ($2 \times 10^7$ cells) and lymphocytes from spleens ($5 \times 10^7$ cells) were injected intravenously into 4-month-old uninfected female NOD mice. Before injection, the recipient mice received 850 rads from a $^{60}Co$ source. They were tested three times a week thereafter for weight loss, polydypsia, and polyuria and were killed 30 days later. Blood glucose was assayed by the glucose oxidase method. Insulin was measured by radioimmunoassay with rat insulin as a standard and A-14 $^{125}I$-labeled bovine insulin as a tracer (*24*). Insulin was extracted from the pancreas in acid ethanol (*24*). The percentage of normal islets of Langerhans present in a pancreas was calculated by counting at least ten islets per pancreas and determining the number not ablated by infiltrating lymphoid cells; pancreatic tissue was fixed in Formalin and stained with hematoxylin and eosin.

| Recipient NOD mouse | Adoptive transfer of lymphocytes | | | |
|---|---|---|---|---|
| | Glucose (mg/dl) | | Insulin (μg/g) | Normal islets (%) |
| | Pre | Post | | |
| | *Uninfected donors* | | | |
| 49 | 100 | 110 | 0.9 | 10 |
| 50 | 145 | 402 | 0.2 | <5 |
| 51 | 145 | 513 | 0.2 | <5 |
| 52 | 150 | 467 | 0.2 | <5 |
| 53 | 146 | 508 | 0.2 | <5 |
| Mean ± SEM | 138 ± 8 | 472 ± 26* | 0.4 ± 0.1 | |
| | *Virus-infected donors* | | | |
| 54 | 134 | 80 | 77.6 | >50 |
| 56 | 133 | 90 | 115.0 | >50 |
| 57 | 154 | 140 | 36.2 | >50 |
| 58 | 117 | 110 | 73.7 | >50 |
| Mean ± SEM | 135 ± 8† | 107 ± 13‡ | 75.6 ± 16‡ | |

*Blood glucose of mice with diabetes (females 50, 51, 52, 53). The value for all members of the group was 400 ± 75. †Not significant. ‡$P < 0.001$.

NOD mice that develop IDDM have pancreatic insulin levels of 0.4 ± 0.1 μg of insulin per gram of pancreas compared to 75.6 ± 16.1 μg of insulin per gram of pancreas in NOD recipients of LCMV-infected lymphocytes ($P < 0.001$). Similarly, fewer than 5% of islet cells from untreated NOD mice are morphologically normal, whereas the majority of islet cells are structurally normal in mice in which the lymphoid cell compartment was reconstituted by lymphocytes from virus-infected NOD mice (Fig. 1).

Viral genes or their expressed products have profound effects on cells. Theoretically, it should be possible to obtain and use such viral products to attack or treat specific cells on the basis of the tropism of the virus (for example, enveloped proteins) or enhancer sequences, enzymes, or other proteins of the virus. In this report, the tropism of a virus for a lymphocyte subset (helper T cells) was used to alter its function. This method abrogates the lymphocyte-caused autoimmune response that leads to severe disease and death. In support of this concept, preliminary data show that LCMV can abort the expected IDDM of BB rats and the T cell

lymphoma of MRL/lpr mice (*16*). A different virus, lactic dehydrogenase virus (LDV), can abort the antinuclear antibody production and immune complex disease of the lupus-prone New Zealand mouse (*17*). LDV is believed to bind to the I-A receptor and replicate in macrophages, hence presumably interfering with antigen processing (*18–20*). Recently, LDV was found to alter significantly the incidence of autoimmune allergic encephalomyelitis in Lewis rats (*21*). Thus, viruses, like bacteria and fungi, or their products, may be used for benefit, and perhaps to treat human illnesses. In addition, viruses may also be used as probes to dissect the molecular basis of various nonvirally caused disorders.


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# Monocyte-Derived Human B-Cell Growth Factor Identified as Interferon-$\beta_2$ (BSF-2, IL-6)


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**Soluble products of either Epstein-Barr virus (EBV)–infected B cells or activated monocytes promote the proliferation of EBV-infected B cells and permit their growth at low cell densities. This suggests that growth factors are important for B-cell immortalization by EBV. In this study, a monocyte-derived factor that promotes the growth of EBV-infected B cells was purified and identified as interferon-$\beta_2$ (IFN-$\beta_2$), which is also known as 26-kilodalton protein, B-cell differentiation factor (BSF-2), and interleukin-6 (IL-6). The purified protein has a specific activity of approximately $4 \times 10^7$ units per milligram of protein in assays of B-cell growth. Thus, IFN-$\beta_2$/BSF-2 is a B-cell growth factor that promotes the proliferation of human B cells infected with EBV.**


THE MECHANISMS OF B-CELL PROliferation, immunoglobulin (Ig) production, and immortalization triggered by Epstein-Barr virus (EBV) are largely unknown. A role for autocrine or paracrine growth factors in these virus-induced processes is suggested by the observation that continuous proliferation of EBV-immortalized human B cells cultured at low cell densities requires the addition of either autologous "conditioned medium" (*1*) or supernatants of activated monocytes (*2*). Autocrine growth factors derived from EBV-infected lymphoblastoid cell lines appear to have functional similarities with T cell–derived "B-cell growth factors" (*3*) or with interleukin-1 (IL-1) (*4*). In contrast, paracrine growth factors derived from activated monocytes appear to be distinct from IL-1 and a variety of other monocyte-derived growth factors (*2*). We sought to identify the monocyte-derived growth factor or factors that permit growth of EBV-immortalized B cells at low cell density. We now report the purification of one such growth factor that we have identified as interferon-$\beta_2$ (IFN-$\beta_2$). This molecule is also known as 26-kD protein, B-cell differentiation factor (BSF-2), and interleukin-6 (IL-6).

The starting material for this purification was the culture medium of human peripheral blood monocytes stimulated with lipopolysaccharide (LPS) and phytohemagglutinin (PHA) (*5*). In a representative experiment we obtained a final purification of $4 \times 10^5$ times the original sample with recovery of approximately 10% of the original biological activity (Table 1).

The purified material was analyzed in reduced conditions by electrophoresis through a 12% polyacrylamide gel (*6*) followed by silver staining (*7*). Two major protein bands were identified with relative mass of 19 and 21 kD (Fig. 1A). This purified material enhanced the proliferation of an indicator lymphoblastoid cell line in a dose-dependent manner and revealed a spe-


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**Table 1.** Representative purification of B-cell growth factor from activated monocyte supernatants. Protein concentration in monocyte supernatant pool, CM-Sephadex pool, and DEAE-Sephacel pool was determined by the method of Smith *et al.* (*18*), whereas the protein content of the Synchropak RP-P pool was determined after silver staining (*7*) by a comparison with a 20-kD protein standard (soybean trypsin inhibitor). One unit of activity is arbitrarily defined as the activity inducing one-half maximal proliferation of an indicator lymphoblastoid cell line (TI) obtained by EBV (B95-8) immortalization of normal peripheral blood B cells. This cell line (in exponential growth phase) was cultured for 2 days in RPMI 1640 medium supplemented with 10% FCS, 2 m*M* L-glutamine, and 5 μg of gentamicin per milliliter at a cell density of $25 \times 10^3$ to $50 \times 10^3$ cells per milliliter. At higher cell densities little or no induction of proliferation was observed.

| Fraction | Total proteins (mg) | Total activity (units × $10^4$) | Specific activity (U/mg) |
|---|---|---|---|
| Monocyte supernatant pool | 84 | 2.4 | $2.86 \times 10^2$ |
| CM-Sephadex pool | 46 | 28.2 | $6.0 \times 10^3$ |
| DEAE-Sephacel pool | 1.8 | 8.96 | $4.87 \times 10^4$ |
| Synchropak RP-P pool | $6 \times 10^{-4}$ | 2.4 | $4.0 \times 10^7$ |