shown in Table 1, control samples are expressed as a relative migration ratio of 1.0. Treatment with bleomycin (100 μg/ml) for 1 hour results in damage to the DNA and a decrease in the relative migration of the nucleoids to 0.61. One hour after recovery from bleomycin treatment, the migration ratio has returned to 1.0, consistent with the concept that the DNA damage has been repaired. A similar result is obtained when the inactive analog W12 is added to the recovery medium. In contrast, no repair is observed in cultures to which W13 (30 μg/ml) was added during the 1-hour recovery period. These samples have a relative migration ratio of 0.57, which is similar to the samples treated with bleomycin but not allowed to recover. Such data indicate that W13 inhibits DNA repair and that this may be the mechanism by which it increases bleomycin lethality and inhibits recovery from bleomycin-induced potentially lethal damage.

The studies described were predicated on the involvement of calmodulin in cell proliferation and were designed to help elucidate its role in this process. The finding that calmodulin appears to be involved in DNA repair is compatible with its role in the G_1-S transition, since cells must repair damage to their DNA before successful replication can proceed. If calmodulin participates in DNA repair, then some of the enzymes involved in purine and pyrimidine metabolism, as well as other proteins involved in DNA repair, must be regulated by calmodulin. Identification of these proteins and elucidation of their specific roles in DNA repair are worthy of future investigations.

JAMES G. CHAFOULEAS
WADE E. BOLTON
ANTHONY R. MEANS
Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

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12 March 1984; accepted 18 April 1984

Autoantibodies to a 64-Kilodalton Islet Cell Protein Precede the Onset of Spontaneous Diabetes in the BB Rat

Abstract. *Spontaneous insulin-dependent diabetes mellitus (IDDM) in the BB rat is associated with the presence of antibodies to a 64-kilodalton rat islet cell protein. These protein antibodies appeared in young animals and remained for as long as 8 weeks before the clinical onset of IDDM. Antibodies to a 64-kilodalton human islet cell protein were found to be associated with human IDDM. Detection of the antibodies may therefore be used to predict an early immune reaction against pancreatic B cells.*

Insulin-dependent diabetes mellitus (IDDM) in humans develops after a specific reduction of pancreatic B cells leading to insulin deficiency and inability to control glucose metabolism. The clinical onset is accompanied by a number of immunopathological phenomena (*1*), in particular the occurrence of autoantibodies that react with antigenic determinants present in the cytoplasmic compartment as well as in the plasma membrane of the B cells (*2*). Earlier we showed that sera from newly diagnosed IDDM patients, containing islet cell surface antibodies (ICSA's) to B cells, have autoantibodies to a 64-kilodalton (kD) human islet cell protein (*3*). Evidence suggests that human IDDM has a long prodromal period, and islet cell antibodies have been detected in individuals 2 to 8 years before the clinical presentation of diabetes (*4*). This premonitory autoimmune response is difficult to study in humans because susceptible individuals are not easily identified. However the BB rat, which spontaneously develops an IDDM analogous to that of humans (*5*), is amenable for prospective analysis. We have now examined whether rat islet cell proteins are specifically recognized by antibodies in BB rats before and after diagnosis of IDDM.

Serum samples were obtained from two different colonies of BB rats (*6*). In one colony of outbred animals (BB/Ontario), housed at the Laboratory of Animal Resources in Ottawa, Ontario, rats from two separate lines were studied. Animals from one line had a high incidence of IDDM (60 to 70 percent by 60 to 120 days of age), and animals from the other had a low incidence of IDDM (< 5 percent). The second colony consisted of rats originating from the University of Massachusetts in Worcester that had been kept in sister-brother matings for 10 to 16 generations; this colony was housed in our laboratory. Animals in one of these lines (BB/Hagedorn) had a high incidence of IDDM (80 to 90 percent by 60 to 120 days of age), while those in another line (the BB w-subline, also from Worcester) in which IDDM did not occur in more than ten generations of brother-sister matings served as controls. ICSA's were determined by assay with ^{125}I-labeled protein A (*7*) with a cell line (RIN-5F) developed from a transplantable rat insulinoma (*8*). Wistar rat islets were labeled with [^{35}S]methionine and lysed with detergent; the lysates were subjected to immunoprecipitation with sera from animals from the two high-incidence groups and their controls as well as from normal Wistar rats (*9*).

Immunoprecipitation with sera from seven BB/Hagedorn rats obtained 1 to 7 weeks before or at the time of clinical diagnosis of IDDM gave a protein that, by comparison with marker molecules, had a molecular size of 64 kD (Fig. 1, lanes d through j). This protein was not detected in fractions immunoprecipitated with sera from three BB rats of the control BB w-subline (Fig. 1, lanes a, b, and c). The 64-kD component was also specifically detected in rat islet cell proteins (Fig. 2, lanes d, e, and f) and transplantable rat insulinoma cell proteins (Fig. 2, lane g) precipitated with sera from high-incidence BB/Ontario rats, but this component was not detected in proteins precipitated with sera from low-incidence BB/Ontario rats (Fig. 2, lanes a, b, and c) or from outbred normal Wistar rats (Fig. 2, lane h).

Major components of 45 kD (probably actin), 55 kD (probably tubulin), and 73 kD (unknown) were precipitated with both BB sera and control sera (Figs. 1

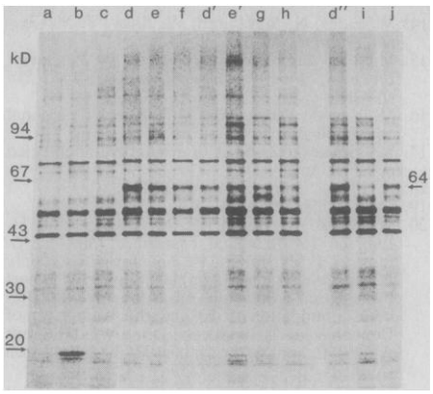

Fig. 1. Immunoprecipitation of Wistar rat islet cell proteins with sera from three control rats of the BB w-subline (not prone to diabetes) (lanes a, b, and c) and with sera from six BB/Hagedorn rats (prone to diabetes) (remaining lanes). Sera from BB/Hagedorn rats were obtained 6 weeks (lanes d and f), 7 weeks (lane e), 4 weeks (lane d'), 2 weeks (lanes e', g, and h), 5 weeks (lane i), and 1 week (lane j) before onset of IDDM. One sample was obtained at the onset of IDDM (lane d'').

and 2). These proteins may represent nonspecifically precipitated cytoskeletal proteins (*10*).

Additional fractions of 105 kD (Fig. 2, lane f), 99 kD (Fig. 1, lanes e' and h, and Fig. 2, lanes d, e, and f), and 90 kD (Fig. 2, lanes d, e, and f) were occasionally observed in proteins immunoprecipitated with sera from high-incidence BB rats. However, these bands were not consistently precipitated with sera from high-incidence BB rats, and they were also seen at low densities in proteins precipitated with control sera. Although antibodies to spleen lymphocytes have been detected in diabetic BB rats (*7*), neither the 64-kD protein nor any other lymphocyte protein was specifically detected in proteins immunoprecipitated with sera from high-incidence BB rats (data not shown).

The 64-kD protein was detected in fractions immunoprecipitated with sera from all BB/Hagedorn rats that developed IDDM (14 of 14) and from 92 percent (11 of 12) of the high-incidence BB/Ontario rats. The incidence of 64-kD antibodies in these rats was different ($P < 0.002$, Fisher's exact test) from that of the corresponding control BB rats of the two colonies (BB w-subline: 5 of 18, 28 percent; low-incidence BB/Ontario: 1 of 8, 13 percent). Sera from eight normal Wistar rats did not contain antibodies to the 64-kD protein (negative sera). The incidence of positive sera in control BB rats was not significantly different from that in normal Wistar rats. Analysis of ICSA's revealed that, compared to the BB w-subline and the low-incidence BB/Ontario line, respectively, 79 percent of the BB/Hagedorn rats (11 of 14) and 92 percent of the high-incidence BB/Ontario rats (11 of 12) were antibody-positive.

In six BB/Hagedorn rats studied prospectively, antibodies to the 64-kD component were present in five of the rats up to several weeks before the onset of IDDM (Fig. 3). Five out of six animals already had the antibodies at 33 or 45 days of age, the earliest age from which a sample was obtained. In one animal (BB rat 2, Fig. 3) antibodies to the 64-kD protein were detected only in a serum sample obtained at the onset of IDDM and 28 days later. At this stage the intensity of the 64-kD band diminished, perhaps because of a lower titer of antibodies (not shown).

Our results show that spontaneously diabetic BB rats in a noninbred colony and in a colony perpetuated in sister-brother matings have autoantibodies that specifically detect a 64-kD islet cell protein. The data suggest that these antibodies occur in animals closely related to the high-incidence BB rats. In humans, the prevalence of islet cell antibodies is also higher among relatives to IDDM patients than in the background population (*11*). The 64-kD rat antigen has the same electrophoretic mobility as the human counterpart detected in human islets with human IDDM sera (*3*). The autoantigens may in fact be identical, since some human IDDM sera precipitate a 64-kD protein from normal rat islets (*12*) and transplantable rat (NEDH) insulinoma cells (*13*). A cross-reactivity between BB rat sera and the 64-kD human islet cell protein has yet to be established.

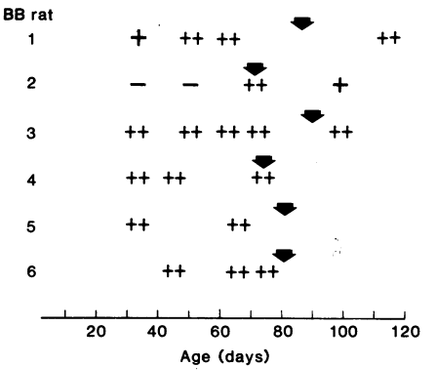

Fig. 3. Prospective analysis of antibodies to the 64-kD antigen. Six BB/Hagedorn rats were observed prospectively from day 33 (BB rats 1 through 5) or day 45 (BB rat 6) until and after the onset of IDDM. Arrows indicate time of onset of IDDM in each animal. At each time point, a strong (++), weak (+), or negative (−) 64-kD band was detected by immunoprecipitation and subsequent sodium dodecyl sulfate–polyacrylamide electrophoretic analyses and autoradiography.

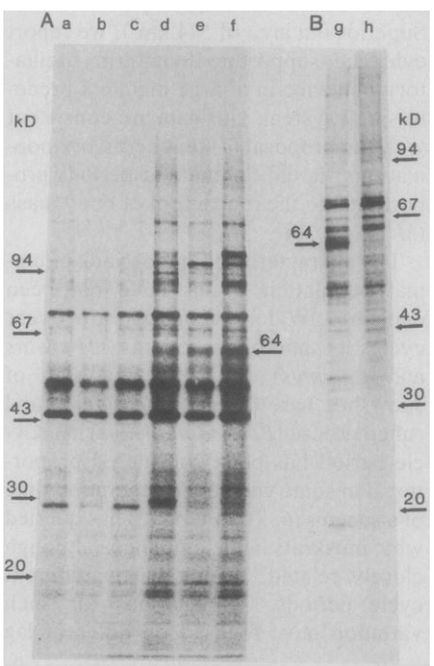

Fig. 2. Immunoprecipitation of Wistar rat islet cell (A) and transplantable rat (NEDH) insulinoma cell (B) proteins with sera from the BB/Ontario rat colony. Sera were obtained from three rats of the low-incidence line (lanes a, b, and c), none of which had any morphologic or metabolic evidence of diabetes; from four rats of the high-incidence line, one of which had impaired glucose tolerance (lane d) and three of which had insulitis (lanes e, f, and g); and from one normal Wistar rat (lane h).

In human IDDM, sera containing both ICSA's and islet cell cytoplasmic antibodies immunoprecipitated the 64-kD antigen. ICSA's in these sera were found to be specific for pancreatic B cells (*3*). In the BB rat, only ICSA's (*7*) but no islet cell cytoplasmic antibodies have been detected (*5*, *14*). Indirect immunofluorescence analyses of BB rat sera tested on B and non–B cells separated by cell sorting (*15*) suggest that ICSA's in sera positive for the 64-kD protein are B cell–specific (*16*). Together with the detection of the 64-kD protein in transplantable rat insulinoma cells, this indicates that the protein is a B cell–specific islet cell protein that is recognized by ICSA's in the BB rat as well as in human IDDM patients.

Our observations of autoantibodies to the 64-kD component in the BB rat provide additional evidence that the spontaneous IDDM of the BB rat is similar to that of humans (*5*). This is important because the BB rat provides a model for the elucidation of prodromal events responsible for B cell destruction. A prospective analysis in diabetes-susceptible BB rats showed the presence of ICSA's and lymphocyte antibodies 40 to 70 days before onset (*7*). Our discovery that antibodies against a putative B cell–specific

protein are present before the onset of diabetes in the BB rat is therefore of particular interest. Earlier we observed that antibodies to the 64-kD human islet cell component were present 2 years before clinical onset of IDDM in a twin brother of an IDDM patient. At that time islet cell cytoplasmic antibodies were still negative, and an increase in glycosylated hemoglobin A_{1c} was yet to be detected (*17*). We therefore speculate that the 64-kD component is a major target antigen in an immunopathological process that culminates in a loss of pancreatic B cells to such an extent that fasting blood sugar can no longer be controlled. Recent evidence from studies of the BB rat (*18*) suggests that immune intervention with cyclosporin A allows prevention of IDDM, provided that therapy was initiated before the onset of IDDM. This discovery has already been used to direct a similar attempt to prevent IDDM in humans (*19*).

STEINUNN BAEKKESKOV
THOMAS DYRBERG
ÅKE LERNMARK
Hagedorn Research Laboratory, DK-2820 Gentofte, Denmark

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10 February 1984; accepted 23 March 1984

Wolves, Moose, and the Allometry of Population Cycles

Abstract. *After a decade of dramatic population fluctuations, protected populations of wolves and moose in Isle Royale National Park in Lake Superior returned in 1983 to the levels observed in the 1950's. Inherent lags in this predator-prey system and the strong recovery of the moose population following a wolf population crash suggest that these populations may continue to cycle with a period length of about 38 (95 percent confidence interval, ±13) years. Such a long-term cycle is consistent with the proposal that period length of herbivore population cycles will characteristically scale according to the fourth root of body mass, a basic allometric relation linking physiological cycles to population processes.*

From long-term studies (*1–3*) of protected wolves (*Canis lupus*) and moose (*Alces alces*) on Isle Royale in Lake Superior (an area of 544 km^2), we report evidence supporting long-term oscillatory behavior in a large mammal predator-prey system. Our data are consistent with the proposal (*4*) that herbivore populations should fluctuate at periods proportional to the fourth root of body mass ($M^{1/4}$).

The characteristic time periods of animal population cycles have not been explained. Widely recognized are 4-year cycles of microtine rodents (*Microtus* and *Lemmus*) and 10-year cycles of snowshoe hare (*Lepus americanus*) and ruffed grouse (*Bonasa umbellus*) (*5*). Cycle period has been considered proportional in some way to the generation time of a species (*6, 7*). Finerty (*5*) has queried why muskrats and lemmings, although closely related, exhibit vastly different cycle periods. Explanations for such variation have focused on different lag times for recovery of woody and herbaceous forage (*8, 9*).

It may seem obvious that large, long-lived mammals would fluctuate at longer intervals than smaller species, but the relation between body size and life cycles is so simple and pervasive that it can be overlooked (*10*). The dependence of cycle period on body size follows logically from the premise that animal cycles reflect the interaction of natality, mortality, and dispersal (*11*), coupled with the allometric scaling of physiological functions and life history characteristics to $M^{1/4}$ (*12–14*).

After wolves colonized Isle Royale in the late 1940's, their numbers stabilized, then fluctuated dramatically, culminating in a population crash in 1980 to 1982 from malnutrition and intraspecific killing. Their population returned in 1983 to the level observed 27 years earlier when first surveyed (*1*). The available data set suggests a period length of about 30 years for this fluctuation, which tracked

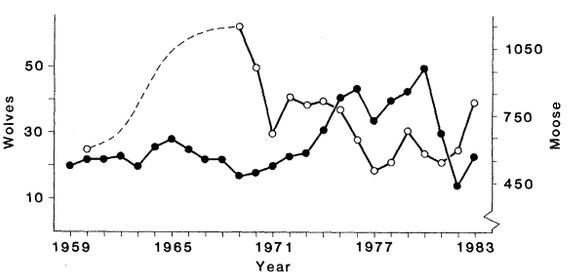

Fig. 1. Wolf (●) and moose population (○) fluctuations on Isle Royale, 1959 through 1983 (*2, 28*).