

7. G. S. Hotamisligil, A. Budavari, D. L. Murray, B. M. Spiegelman, *ibid.* **94**, 1543 (1994).
8. M. F. White and C. R. Kahn, *J. Biol. Chem.* **269**, 1 (1994).
9. G. S. Hotamisligil, D. L. Murray, L. N. Choy, B. M. Spiegelman, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 4854 (1994).
10. R. Feinstein, H. Kanety, M. Z. Papa, B. Lunenfeld, A. Karasik, *J. Biol. Chem.* **268**, 26055 (1993).
11. N. Begum, *Diabetes* **44**, 140A (1995); G. Kroder, M. Kellerer, B. Bossenmaier, A. Mühlhöfer, H.-U. Harig, *ibid.*, p. 264A.
12. G. S. Hotamisligil, P. Peraldi, B. M. Spiegelman, unpublished observations.
13. J. Vilcek and T. H. Lee, *J. Biol. Chem.* **266**, 7313 (1991).
14. J. F. Tanti, T. Gremeaux, E. Van Obberghen, Y. Le Marchand-Brustel, *ibid.* **269**, 6051 (1994).
15. L.-M. Wang *et al.*, *EMBO J.* **11**, 4899 (1992); L.-M. Wang *et al.*, *Science* **261**, 1591 (1993); X. L. Sun *et al.*, *Nature* **377**, 173 (1995).
16. J. M. Stephens and P. H. Pekala, *J. Biol. Chem.* **266**, 21839 (1991).
17. R. G. Douglas *et al.*, *Am. J. Physiol.* **261**, 606 (1991); T. Van Der Poll *et al.*, *ibid.*, p. E457; C. H. Lang, C. Dobrescu, G. J. Bagby, *Endocrinology* **130**, 43 (1992).
18. J. M. Backer *et al.*, *J. Biol. Chem.* **268**, 8204 (1993); T. J. O'Neill, A. Craparo, T. A. Gustafson, *Mol. Cell. Biol.* **14**, 6433 (1994).
19. After complete differentiation, adipocytes were treated with murine TNF- α (3 ng/ml; Genzyme) for 4 days in Dulbecco's modified Eagle's medium (DMEM) containing fetal calf serum (10%) and insulin (100 ng/ml; Sigma). On day 4, cells were washed twice in phosphate-buffered saline (PBS) and incubated in serum-free DMEM containing the same dose of TNF- α for 16 hours. The medium was then changed to phosphate-free medium with TNF- α but without serum. After 1 hour of incubation in this medium, [32 P]orthophosphate (3 mCi/ml, 6000 Ci/mmol; New England Nuclear) was added and incubation was continued for 3 hours at 37°C. Insulin stimulations were done by adding insulin (100 ng/ml) directly to the medium. Plates were washed with ice-cold PBS once and protein extracts were prepared as described. Immunoprecipitations were done from control and TNF- α -treated cells with polyclonal antibodies to IR and IRS-1 and a monoclonal antibody to phosphotyrosine in a 1:100 (v/v) dilution for 4 hours and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and autoradiography.
20. W. J. Boyle, P. van der Geer, T. Hunter, *Methods Enzymol.* **201**, 110 (1991).
21. Specific pathogen-free, male Zucker obese (fa/fa) rats and their lean controls (+/?) were purchased from Charles River Laboratories. On arrival, rats were housed for at least 1 week before the experimental

procedures were begun and fed standard rodent chow and water ad libitum. After a 6-hour fast, rats were anesthetized with ketamine and xylazine (intramuscular), the abdominal cavity was opened, and fat (epididymal), muscle (hind limb), and liver tissues were removed and frozen immediately in liquid nitrogen. Frozen tissues were ground into a fine powder with a mortar and pestle and then homogenized with a Polytron homogenizer at 4°C in 6 $\times$ the tissue volume of homogenization buffer [1% Triton X-100, 150 mM NaCl, 10 mM Hepes (pH 7.4), 250 mM sucrose, 10 mM EDTA, 10 mM EGTA, 10 mM benzamide, 2 mM phenylmethylsulfonyl fluoride (PMSF), and aprotinin (100 μ g/ml)]. All extracts were centrifuged at 12,000 rpm for 30 min at 4°C, and the supernatants were collected for further analysis. All animal procedures were in accordance with institutional guidelines.

22. We thank C. Carpenter for phosphoamino acid analysis, M. Myers Jr. and B. Cheatham for gifts of 32D cells and other reagents, and C. R. Kahn for reviewing the manuscript. Supported by a grant from the National Institutes of Health (DK 42539 to B.M.S.) and fellowships from the American Diabetes Association (G.S.H.) and from the Association pour la Recherche sur le Cancer and Juveniles Diabetes Foundation International (P.P.).

20 July 1995; accepted 31 October 1995

TECHNICAL COMMENTS

The Age of the Waiho Loop Glacial Event

George H. Denton and C. H. Hendy (1) cite evidence from a South Pacific island for global forcing of Younger Dryas climatic cooling. However, their interpretation of the data can be challenged; more likely, earlier interpretations (2) that the Franz Josef Glacier in New Zealand's Southern Alps advanced before Younger Dryas time are still valid.

Denton and Hendy derive a simple weighted mean age of $11,150 \pm 14$ ^{14}C years before present (B.P.) from 36 radiocarbon dates and accept this without statistical testing. Following recommended procedures (3, 4) a mean age of $11,130 \pm 20$ ^{14}C years B.P. can be calculated, but applying the χ^2 test for comparing multiple radiocarbon dates (3, 4) shows this is not a valid age. By rejecting those dates with the largest individual χ^2 values, a statistically more valid weighted mean age of $11,180 \pm 20$ can be calculated.

Denton and Hendy (1) allow for about 100 years of transport time before wood deposition by the glacier, and they reject earlier evidence of a local origin for the wood (2). Their model requires that a deposit with abundant wood fragments, some with bark attached (2), survived about 8 km of supraglacial transport in a high rainfall environment. This is highly unlikely, and a local origin for the wood would be more parsimonious. Furthermore, even if their model is accepted,

allowing 100 years for transport is overly generous. At a modest glacier flow rate of 1 m per day, it would take only about 20 years for the wood to travel the suggested 8 km. Thus, it is more likely that the glacier deposited the dated material before 11,100 years B.P., probably around 11,150 to 11,180 ^{14}C years B.P.

Denton and Hendy suggest the Waiho Loop advance would have been widely represented in other valleys. However, no correlative of the Waiho Loop moraine is known from the western parts of the island (5). The physiographic setting of the moraine is not unique, and numerous valleys to the north and south should contain equivalent moraines, had they been deposited. This suggests it was a local event and not representative of a widespread advance of glaciers in the Southern Alps. This is not unusual, as in a similar setting in southern Chile, a glacier has advanced, but not surged, over 10 km down Seno Exmouth since 1940, while adjacent glaciers have been retreating.

Direct correlation of the Waiho Loop advance with initiation of Younger Dryas cooling is invalid as the climate change that led to the advance would have occurred before the advance itself, and thus before the initiation of Younger Dryas cooling. The most accurately dated direct evidence (6) for climatic oscillations around 11,000 ^{14}C years B.P. shows [con-

verting calendar ages to ^{14}C ages (4)] that a pre-Younger Dryas warm peak occurred at around 11,100 ^{14}C years B.P., before full Younger Dryas conditions set in by 10,800 ^{14}C years B.P. Using the age of 11,180 ^{14}C years B.P., one can only conclude that the Waiho Loop glacial event and the climatic changes that triggered it were not coincident with Younger Dryas climate cooling in the North Atlantic region.

The interpretation of Denton and Hendy of a Younger Dryas age advance of Franz Josef Glacier is unwarranted and earlier suggestions (2) that the Waiho Loop moraine was constructed before Younger Dryas time remain the most secure interpretation of the data. Evidence from this site should not be used to demonstrate global forcing of the Younger Dryas cooling event.

M. C. G. Mabin

Department of Tropical Environment Studies
and Geography,
James Cook University of
North Queensland,
Townsville, Q4811, Australia

REFERENCES

1. G. H. Denton and C. H. Hendy, *Science* **264**, 1434 (1994).
2. J. H. Mercer, *N.Z. Geol. Geophys.* **31**, 95 (1988); P. Wardle, *N.Z. Bot.* **16**, 147 (1978).
3. G. K. Ward and S. R. Wilson, *Archaeometry* **20**, 19 (1978).
4. M. Stuiver and P. J. Reimer, *Radiocarbon* **31**, 215 (1993).
5. C. J. Burrows, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **27**, 287 (1979); A. F. Gellatly, T. J. H. Chinn, F. Rothlisberger, *Quat. Sci. Rev.* **7**, 227 (1988).
6. S. J. Johnsen *et al.*, *Nature* **359**, 311 (1992); R. B. Alley *et al.*, *ibid.* **362**, 527 (1993).

30 June 1995; accepted 22 August 1995

Response: Two issues are at the core of any attempt to correlate glacial events between the Northern and Southern hemispheres: (i) the resolution of the available age control, and (ii) whether a specific dated moraine (or moraines) is representative of a regional glacial system. Within the context of addressing these issues, we make the following points.

For the origin of the radiocarbon (^{14}C)-dated wood samples at Canavan Knob, Mabin, favors the earlier suggestions of Mercer (1) and Wardle (2), who thought the wood was in-situ because some pieces appeared to be rooted in place. From extensive excavations, we concluded in our report (3) that none of the wood in the quarry section at Canavan Knob was so rooted. We suggested (3) that a value of 100 years was reasonable for wood transport from the inner valley wall to Canavan Knob, first by mass wasting and then on the glacier surface. If we are correct that the wood was derived from the inner valley, then Mabin would seem to favor a supraglacial transit time of about 20 years to Canavan Knob. Because it does not include time for wood deposition on the glacier surface, this estimate would have the effect of increasing our estimate of the age of the Canavan Knob wood bed by less than 80 years. We favor our own estimate, but we cannot be sure which value is more accurate because the late-glacial flow rates of Franz Josef Glacier are unknown. Given the probable variations in the interhemispheric exchange rate of atmospheric CO_2 and differences in oceanic-atmospheric exchange rates in the two hemispheres, detection of so small a difference for paleoclimatic events in opposite sectors of the globe is probably beyond the resolution of ^{14}C dating. Also, the stated errors measure only the precision of the data and do not reflect the overall range of error associated with ^{14}C dating.

The procedure that we used to determine the age of wood in the basal diamicton at Canavan Knob is based on that of Ward and Wilson (4) and Wilson and Ward (5), as set out in Gupta and Polach (6). The several replicates of individual samples were reduced to error weighted means. Then the assemblage of 25 discrete dates of wood was reduced to an error weighted mean age, which would be valid if the wood samples were contemporary. Mabin is correct in that the χ^2 test shows that the distribution lies just outside that for contemporary material. As the number of dates to be compared is increased, the T value increases faster than χ^2 . When more than 15 dates are pooled, χ^2 exceeds T and the standard deviation (SD) of the error weighted mean becomes smaller than the actual spread of the data. However, this is

not unexpected because the individual wood samples were not strictly contemporaneous, as shown by the age spread of several decades for modern wood now on the Franz Josef Glacier. Our value of 100 years for mass wasting and glacier transport is far greater than the SD derived from the error weighting of the 25 dates, and greater than the spread of the alternative mean ages suggested by Mabin. Given this built-in uncertainty, there seems little virtue in attempting to establish a more "precise" mean age with a difference of only a few decades.

The case for a Younger Dryas event in New Zealand would be strengthened if more late-glacial moraines yielded dates between 10,000 and 11,050 ^{14}C years B.P. But contrary to Mabin's contention, late-glacial moraines could not have been deposited in most valleys northeast and southwest of Franz Josef Glacier in the same physiographic position at the Waiho Loop because this glacier has a large accumulation area feeding a narrow tongue that consequently extends to low elevation near the steep mountain front. Therefore, in response to typical values for late-glacial snowline lowering of 250 to 500 m (7), the glacier would have advanced beyond the mountain front and spread out onto the flattish coastal terrain as a piedmont lobe. Most other west-facing glaciers are smaller than the Franz Josef and now terminate well behind the mountain front. Even after expanding in response to this magnitude of late-glacial snowline lowering, these glaciers would still have terminated in steep valleys behind the mountain front, where only a few moraine remnants could have survived since late-glacial time. One such remnant discovered by Basher and McSeveney (8), well behind the mountain front in a heavily vegetated valley near the head of Cropp River, yielded a date of 10,250 ^{14}C years B.P. Five other wood samples collected from this moraine remnant by C. Schlüchter and G. Denton afforded an error weighted mean age of $10,055 \pm 29$ ^{14}C years B.P. Hence the Cropp River moraine remnant falls within latest Younger Dryas time.

We agree that the climatic change that caused the late-glacial advance of the Franz Josef Glacier must have occurred before the advance itself. But this situation applies to all paleoclimatic indicators, including those used to register the ^{14}C -dated evidence of Younger Dryas cooling in Europe and the North Atlantic Ocean. We know of no reason why the response of this glacier to late-glacial climatic change would have been much different from that of European glacial and vegetation systems. In fact, the Franz Josef may have responded more quickly. However, the slightly differing re-

sponse rates could not be discerned with ^{14}C dating.

We pointed out that previous dates of wood from the lowest Canavan Knob diamicton were older than those that we measured (3). We dated a total of 25 discrete samples [including, we believe, the same piece of wood Mercer (2) sampled] so as not to overlook old wood. Therefore, we disagree with Mabin that the earlier age interpretation is more valid than our new estimate.

For interhemispheric correlation of Younger Dryas cooling, we would not use a ^{14}C time scale that comes from converting the calendar year chronology of Greenland ice cores because the tree-ring calibration extends back only 10,000 years (9). We used the best ^{14}C -dated paleoclimatic records for Younger Dryas cooling in Europe and the North Atlantic Ocean (10, 11). Since publication of our report (3), new ^{14}C dates obtained by accelerator mass spectrometry afford an error weighted mean age of $11,230 \pm 40$ ^{14}C years B.P. for the Laacher See Tephra in Switzerland (12). From varve counts in lacustrine cores, it is known that the Laacher See Tephra is about 200 calendar years older than the distinctive isotopic and pollen-spectra changes at the beginning of Younger Dryas time (12). If it is assumed that the length of radiocarbon and calendar years had a one-to-one ratio at that time, then the Younger Dryas would have begun in Switzerland at about 11,030 ^{14}C years B.P. This brings the Swiss chronology into line with that of northern Europe (10, 11). Overall, these results suggest that Younger Dryas cooling in Europe and the North Atlantic Ocean occurred at about 11,000 to 11,050 ^{14}C years B.P., close to the age of the Franz Josef advance over Canavan Knob to the Waiho Loop. Consequently, it remains our opinion that these events in opposite sectors of the globe were coeval within the range of error associated with ^{14}C dating.

G. H. Denton

Department of Geological Sciences and
Institute for Quaternary Studies,
University of Maine,
Orono, ME 04469, USA

C. H. Hendy

Department of Chemistry,
University of Waikato,
Private Bag 3105, Hamilton, New Zealand

REFERENCES

1. J. H. Mercer, *N.Z. Geol. Geophysics* **31**, 95 (1988).
2. P. Wardle, *N.Z. Bot.* **11**, 349 (1973); P. Wardle, *ibid.* **16**, 147 (1978).
3. G. H. Denton and C. H. Hendy, *Science* **264**, 1434 (1994).
4. G. K. Ward and S. R. Wilson, *Archaeometry* **20**, 19 (1978).
5. S. R. Wilson and G. K. Ward, *ibid.* **23**, 19 (1981).
6. S. K. Gupta and H. D. Polach, *Radiocarbon Dating*

Practices at ANU (Radiocarbon Laboratory, Research School of Pacific Studies, Australian National University, Canberra, Australia, 1985).

7. S. C. Porter, *Quat. Res.* **5**, 27 (1975); M. Meisch, *Occasional Publications* **3** (Geographisches Institut, Universität Zürich, Zürich, Switzerland, 1981).

8. L. R. Basher and M. McSeveney, *R. Soc. N.Z.* **19**,

263 (1989).

9. B. Kromer and B. Becker, *Radiocarbon* **35**, 125 (1993).

10. J. Mangerud, B. G. Andersen, E. Larsen, *Terminat. Pleistocene* **5**, 1 (1992).

11. S. Björck and P. Möller, *Quat. Res.* **28**, 1 (1987); S. J. Lehman and L. D. Keigwin, *Nature* **356**, 757 (1992);

J. C. Duplessy, in *Global Changes of the Past*, R. S. Bradley, Ed. (University Center for Atmospheric Research, Office for Interdisciplinary Earth Studies, Denver, CO, 1989), pp. 341–355.

12. I. Hajdas et al., *Radiocarbon*, in press.

25 July 1995; accepted 22 August 1995

Plasma Viral Load, CD4⁺ Cell Counts, and HIV-1 Production by Cells

Recent papers have reported large amounts of human immunodeficiency virus (HIV) particles circulating in the blood of infected patients (1–3). In the total blood volume, the number of virions can equal 10⁶ particles per milliliter, or an estimated 10⁹ HIV particles per milliliter (1, 2). In his article, John M. Coffin refers (4, p. 483) to an “extraordinarily large number of replication cycles that occur during [HIV] infection of a single individual.” But retrovirus-infected cells, in general, produce large amounts of progeny virions, and most of these viruses are not infectious (5). Thus, the importance of the quantity of virus, both infectious and uninfected, circulating in the blood should not be overemphasized. The most important question raised from these papers is how many cells in the body are needed to produce the billion virus particles observed in the symptomatic patients.

To address this issue, we cultivated for up to 3 days human T cells of the HUT78 line that were chronically infected with the HIV-1_{SF2} strain. This virus strain replicates with moderate kinetics in this T cell line. We monitored viable cell number with the use of trypan blue dye exclusion and determined the percent of productively infected cells with the use of indirect immunofluorescence assays (IFA) on fixed cells (6). Amounts of virus were measured by branched DNA with the use of a kit provided by Chiron Corporation (Emeryville, California).

The HUT78 T cells expressed HIV proteins as detected by IFA. After initial trypsinization to remove virions associated with the cell surface (7), we measured virus production by 2 million of these cells cultured in 2 ml of medium. Within 24 hours, we detected about 120 million viral RNA genome copies which means that virus production by 2 × 10⁶ cells is about 30 virions per cell (that is, two RNA molecules per virion). After 48 hours the amount of virus production rose to 400 million RNA molecules, or about 100 particles per cell.

Because the initial studies on virus production used an established transformed T cell line (HUT78), we also examined virus production by infected peripheral blood mononuclear cells (PBMC), the major target of HIV. We acutely infected cultured

PBMCs with either the highly cytopathic HIV-1_{SF33} strain or a molecular clone of the less cytopathic HIV-1_{SF2} strain. Cell culture fluids were changed daily. On the fifth day, when reverse transcriptase activity (~400,000 cpm/ml) in the cell culture fluids was high, the supernatants were collected, filtered, and assayed for viral RNA with the use of the branched DNA technique. The infected PBMCs were trypsinized to remove and inactivate cell-associated virus (7), monitored for cell viability and expression of viral proteins as detected by IFA, and plated as infectious centers on PHA-stimulated normal PBMCs. The dilutions involved duplicate cultures receiving 1000, 500, 100, 50, and 10 cells per well. At each dilution of cells, we performed the trypsinization procedure to eliminate any virus spread that might have occurred during the dilution.

We found that 10 to 15% of the PBMC showed HIV protein expression by both assays. Viral RNA production in 2 ml of culture fluid indicated the release of approximately 125 to 200 particles per cell in 24 hours (Table 1), which is similar to our result with the HUT78 cells. Studies by Dimitrov et al., which used cultured HIV-1-infected CEM cells (8), showed that at the time of peak virus production 1000 particles were released by an infected cell. These data and ours demonstrate the large capacity for virus release by one infected cell and suggest that only about 10

million cells would be needed to maintain the billion virus particles observed in the plasma of some subjects (1, 2). Possibly even fewer cells are required, given the limits of the in vitro studies conducted.

These findings on free virus production do not include the number of virions that remain cell-associated. Such viruses must be in much greater number than the free virus released, as they are easily detected as budding particles by electron microscopy, and as they can spread 100- to 1000-fold more effectively than can free virus (8). These observations underline the importance of combating the cellular source of HIV and not just de novo infection by its viral progeny (9).

Finally, the immediate rise in the CD4⁺ cell counts after antiretroviral therapy may reflect a response to the drug itself and not to a drug-induced protection from CD4⁺ cell death, as hypothesized in other studies (1, 2). Records from one individual provide valuable data. Within 30 minutes after a needle stick injury, this person was given 3' azido-3 deoxythymidine (AZT) prophylaxis (1200 mg/day for 2 days, 1000 mg/day for 12 days, and then 500 mg/day for 2 weeks). He has been shown not to be infected. Total lymphocyte and CD4⁺ and CD8⁺ cell counts were conducted on a regular basis and examined in relationship to the pre- and post-drug therapy measures. Within 48 hours after AZT treatment, both lymphocyte and CD4⁺ cell counts rose substantially from the baseline amount (Fig. 1). They increased by 30% by day 12 and remained at this high count throughout the course of

Table 1. HIV-1 replication in peripheral blood mononuclear cells. Data obtained from viral RNA measurements in 2 ml of fluid after culture of 2 × 10⁶ infected PBMC for 24 hours. Number of cells releasing virus are estimated from percent viability, data from infectious center, and immunofluorescence assays. Computations do not include cells that may have died after virus release (for example, with HIV-1_{SF33}).

HIV-1 isolate	Cells releasing HIV-1	Viral RNA genome copies per milliliter	Viral particles per cell
SF33	1.4 × 10 ⁵	32 × 10 ⁶	228
SF2	2.4 × 10 ⁵	30 × 10 ⁶	125

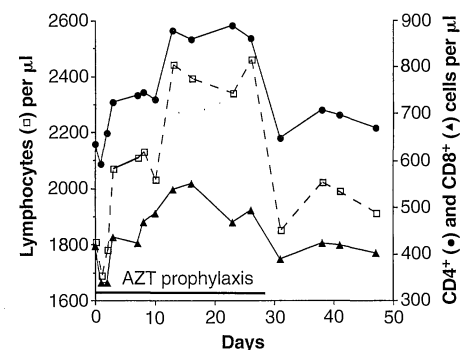


Fig. 1. Lymphocyte counts during and after prophylactic treatment of a healthy individual with AZT after an HIV needle stick injury. Zero represents time period just before initiation of therapy. The y axis on the left shows a total lymphocyte count; the y axis on the right shows a count for specific cell types.