T_H1 cells prompted us to focus our attention on the IFN-γ receptor (IFN-γR). This receptor is composed of two chains, IFN-γRα and the recently cloned accessory factor–1 (AF-1) (also referred to as IFN-γRβ) (*18*). Although IFN-γRα can, by itself, bind IFN-γ with high affinity (*19*), interaction of this chain with AF-1 is required for IFN-γ–mediated signaling, including the activation of STF-IFNγ and the induction of IRF-1 gene expression (*20*). We therefore examined the T_H1 and T_H2 clones for the expression of these receptor components. Fluorescence-activated cell sorting (FACS) analysis of cell surface expression of IFN-γRα (Fig. 4A) revealed that the clones contain roughly equal amounts of IFN-γRα chain. However, when we examined these clones for the presence of AF-1–encoding mRNA by Northern (RNA) analysis (Fig. 4B), we found that the T_H2, but not the T_H1, clone expressed the AF-1 mRNA transcript. Reverse transcription of RNA from three different T_H1 clones followed by the polymerase chain reaction (RT-PCR) confirmed the absence of AF-1 expression in these cells (Fig. 4C). To test whether reintroduction of AF-1 expression could rescue IFN-γ signaling in T_H1 cells, we transiently transfected a complementary DNA (cDNA) encoding AF-1 into a T_H1 clone (D1.1). Transfection of AF-1, but not a mock transfection, led to the appearance of STF-IFNγ in T_H1 cells (Fig. 5). Detection of this complex by EMSA was blocked by an antiserum against Stat1 (Fig. 5).

Our data indicate that T_H1 cells cannot activate the Jak-STAT pathway in response to IFN-γ because the AF-1 component of the IFN-γ receptor is not expressed. Down-regulation of the IFN-γ signaling pathway in T_H1 cells may allow the immune system to selectively inhibit the proliferation of T_H2 cells, while permitting T_H1 cells to escape the antiproliferative effects of the IFN-γ that they secrete. Preliminary data reveal that precursor T helper cells are able to activate STF-IFNγ. If these cells are cultured in the presence of IFN-γ, the resulting T cell population, which is greatly enriched in T_H1 cells, does not activate STF-IFNγ in response to IFN-γ restimulation (*21*). Thus, during differentiation into T_H1 cells, T cells may lose the capacity to activate STF-IFNγ. This finding is consistent with a model in which modulation of cytokine signaling may play an important role in the acquisition of specific T helper cell phenotypes.

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24. Immunoprecipitates were washed twice in kinase buffer (20 mM Hepes, pH 7.4, 2 mM $MnCl_2$, 10 mM $MgCl_2$, 1 mM dithiothreitol, 150 mM NaCl, 0.1 mM sodium orthovanadate, and 0.4 mM phenylmethylsulfonyl fluoride), then resuspended in 40 μl of kinase buffer and subjected to an in vitro kinase reaction with [γ-^{32}P]adenosine triphosphate (ATP) as previously described [O. Colamonici, H. Uyttendaele, P. Domanski, H. Yan, J. Krowleski, *J. Biol. Chem.* **269**, 3518 (1994)].
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27. The cDNA was obtained by reverse transcription with the antisense primer 5′-AAT ACT TGT AGC ATC CAG AA-3′. Half of the cDNA was then subjected to PCR amplification with the above primer and the following sense primer: 5′-GAA CAA ATC GAA GAG TAT CT-3′. All PCR was performed with 25 cycles consisting of 1 min at 94°C, 1 min at 44°C, and 1.5 min at 72°C with a 7-min extension at 72°C for the last cycle. DNA amplification products were analyzed by acrylamide gel electrophoresis.
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29. Transient transfections of D1.1 were carried out by electroporation as previously described [J. Lederer, J. Liou, M. Todd, L. Glimcher, A. Lichtman, *J. Immunol.* **152**, 77 (1994)] except that a Cell-Zap II instrument (Andersen System) and 40-μF capacitance were used. Cells were assayed 48 hours after transfection. The constitutive STF-IFNγ DNA binding activity detected in extracts of AF-1–transfected D1.1 is likely to be due to the presence of endogenous IFN-γ secreted by these cells. Addition of a neutralizing IFN-γ antibody to the culture of the AF-1–transfected D1.1 decreased the amount of STF-IFNγ in untreated cells.

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TECHNICAL COMMENTS

Seasonal Precipitation Timing and Ice Core Records

Commenting on our work with isotope tracers and the origin of moisture in general circulation model simulations (*1*), Eric J. Steig *et al.* (*2*) suggest that changes in the seasonal distribution of precipitation may provide strong control on isotopic variability in Greenland ice cores. In principle, we agree with the thrust of their comment. In a broad sense, without consideration of specific processes, the seasonality effects discussed by Steig *et al.* and the moisture source effects described in our report are two classes of the same general phenomenon: evaporation, distillation, and transport of isotopes over different temperature regimes. Although the analysis of Steig *et al.* for Greenland precipitation over the last century suggests that seasonal effects are a significant component of interannual isotopic variability, general circulation models (GCMs) represent one of the few means of assessing the importance of this phenomenon for interpreting the isotopic record over glacial cycles. The GCM approach is important for understanding the relationship between $\delta^{18}O$ and temperature because (i) thermodynamic principles and analysis of modern isotopic data suggest that present-day spatial $\delta^{18}O$-temperature correlations cannot be considered an exact surrogate for the temporal relationship between these variables and (ii) geographic isotopic variability—for example, the differences in isotopic values among ice

cores—can best be examined with a three-dimensional model.

Although we are limited by having only two complete multiyear isotope simulations (ice age and modern), some aspects of temporal isotopic variability over Greenland can be addressed. For example, extending the approach of Steig *et al.* (*2*) to the Goddard Institute for Space Studies (GISS) 4 × 5° resolution isotope tracer model experiments reveals that there is virtually no systematic change in the seasonal timing of Greenland precipitation between the ice age and modern simulations. As a result, the simulated glacial-interglacial change in "precipitation-weighted temperature" is not significantly better correlated with isotopic change over Greenland than is the change in simulated temperature alone. Thus, these model results do not support the suggestion of Steig *et al.* that seasonality is a primary influence on isotopic change between all climates.

Assessing the importance of moisture source variability, as opposed to local air temperature, is complicated because of the wide range of sources—and therefore climate processes—that contribute to Greenland precipitation in the model. It is not our intention to imply that changes in the origin of moisture source can by themselves account for all isotopic variability in Greenland ice cores. For example, in the 8 × 10° resolution model, local air temperature change is well correlated with the glacial-interglacial isotopic change over the Greenland and Laurentide regions (*3*). The same degree of correlation is also apparent in the 4 × 5° resolution model (R^2 values for this correlation range from 0.8 to 0.5, depending on exactly which points are considered). But the 4 × 5° model highlights the fact that different regions have different isotopic "sensitivities" to climate change (Fig. 1). For example, the slope of the glacial-interglacial $\Delta\delta^{18}O/\Delta T$ relationship over North-Central Greenland averages about 0.8 per mil per degrees Celsius, whereas the glacial-interglacial slope over southeastern Greenland is 0.4 per mil per degrees Celsius (the modern spatial relationship is about 0.6 per mil per degrees Celsius). Regression analysis shows that changes in the origin of moisture source can account for some of this difference in isotopic sensitivity, one example being the interplay between North American and North Pacific moisture over Greenland examined in our report (*1*). However, more precise physical explanations for these regional differences may come with additional climate simulations, and more confidence in their significance may come with comparison to other GCMs fitted with tracer diagnostics, such as the Laboratoire de Météorologie Dynamique (Paris) (*4*) and the Hamburg (*5*) models.

Although we recognized that moisture source variability, isotopic variability, and air temperature variability must all be related to some degree, our report focused on the broad-scale climate processes responsible for the ice core isotopic shifts. The existence of multiple moisture sources for Greenland in our three-dimensional general circulation model experiments suggests that other processes, aside from those just involving the North Atlantic ocean, need to be considered. Steig *et al.* propose one variable, sea ice extent, which could be important in shaping the ice core record. Although our model results show no evidence as yet of their specific seasonality mechanism, the results do warrant more detailed investigation into the regional variability of the $\delta^{18}O$-temperature relationship, including the influence of sea ice.

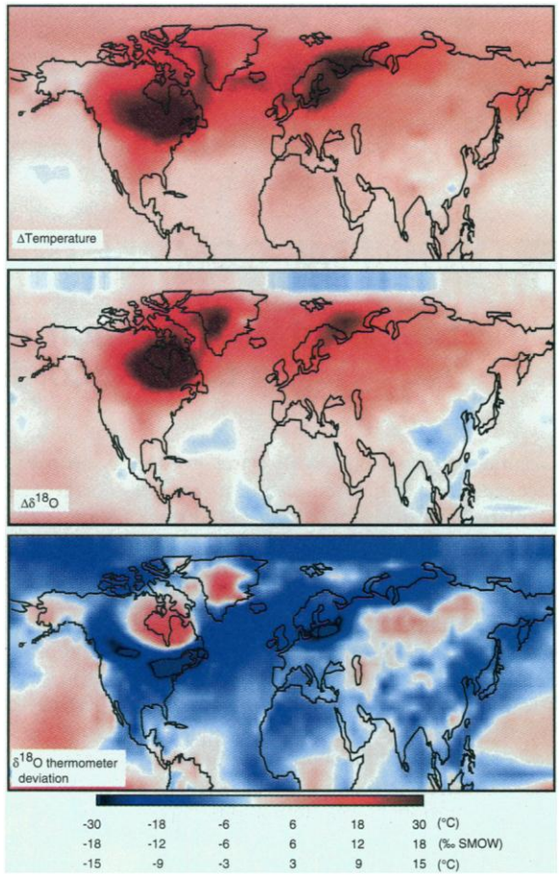

Fig. 1. Predicted mean annual fields for (top two panels) the difference between the glacial maximum and modern (control) simulations for surface air temperature and $\delta^{18}O$; (lower panel) the "error" produced (in degrees Celsius) if the $\delta^{18}O$ change were used to predict the temperature change with a universal scaling of 0.6 per mil per degrees Celsius. Temperature change is underestimated in the blue-shaded regions and overestimated in red-shaded regions.

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