9. Fuel load quantities, divided into standard time-lag size classes (1 hour, 10 hour, 100 hour, and 1000 hour; corresponding to 0 to 0.6, >0.6 to 2.5, >2.5 to 7.6, and >7.6 cm in diameter), fuel height, and leaf litter depth were measured along randomly directed 10-m transects at three points within each plot (125, 250, and 375 m). Additional fuel load measurements were made in the vicinity of all observed fires.
10. At each observation site (n = 44), roughly 10 m of fireline was observed every 30 s for several minutes (average observation time, 7.4 min), and the average minimum and maximum of each flame characteristic were recorded. Flareups of short duration or small area were noted separately.
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14. Leaf fall from damaged trees begins within 2 days of a burn. We have observed contiguous layers of leaf litter in recently burned forests in close proximity to smoldering logs. Frequent observations by local residents that forest stands often burn more than once in a season may be explained by smoldering logs reigniting fires once litter depths become sufficient to carry fire through the stand.
15. The mortality of trees (>10 cm dbh) in previously unburned forests that burned in 1995 was 38% 1 year after the fire and 68% at the end of the second year. Annual mortality in unburned forest during this time period was <1%.
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17. In forest subjected to fire, vines frequently form a dense mat at 30 to 200 cm above the ground, and grasses can form up to 70% of the ground cover.
18. Field studies of forests in 1996 revealed a representative plot with only 18 live trees per hectare (with 302 trees standing dead per hectare). The fires of 1997 had left only three live trees in this plot, which was threatened by an oncoming fire, when recensused.
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29. In Paragominas, burns detected in the imagery were compared with data from landowner questionnaires (n = 75) that described fire history from 1982 to 1995. Questionnaire data included 51.4% of the study region and showed 100% detection of reported fires that occurred within 1 year of the image date. Comparisons between the area reported burned by landowners with data from the imagery classifications showed that the area burned was systematically underreported ($P < 0.001$; sign test) by an average of 43%. Only small fires (<50 ha) were overestimated by landowners.
30. We thank C. Uhl, A. Taylor, G. P. Patil, F. Williams, and four anonymous reviewers for comments on earlier versions of the manuscript. This research was funded by a grant from the PPG7 "Programa de Pesquisa Dirigida" (MMA/MCT/FINEP). Additional financial support was provided by NSF, NASA, and the U.S. Agency for International Development.

16 February 1999; accepted 16 April 1999

The Nature of the Principal Type 1 Interferon–Producing Cells in Human Blood

Frederick P. Siegal,[1*] Norimitsu Kadowaki,[2] Michael Shodell,[3] Patricia A. Fitzgerald-Bocarsly,[4] Kokila Shah,[1] Stephen Ho,[2] Svetlana Antonenko,[2] Yong-Jun Liu[2*]

Interferons (IFNs) are the most important cytokines in antiviral immune responses. "Natural IFN-producing cells" (IPCs) in human blood express CD4 and major histocompatibility complex class II proteins, but have not been isolated and further characterized because of their rarity, rapid apoptosis, and lack of lineage markers. Purified IPCs are here shown to be the $CD4^+CD11c^-$ type 2 dendritic cell precursors (pDC2s), which produce 200 to 1000 times more IFN than other blood cells after microbial challenge. pDC2s are thus an effector cell type of the immune system, critical for antiviral and antitumor immune responses.

Interferons were discovered in the 1950s as factors rapidly produced by virus-infected cells that enable neighboring cells to resist virus infection (*1*). IFN-α (leukocyte IFN) and IFN-β (fibroblast IFN), the two type 1 antiviral IFNs, are distinct from type 2 IFN-γ produced by effector T cells. Specialized leukocytes, the "natural IFN-producing cells" (IPCs), were shown to be the chief IFN-α producers in response to enveloped viruses, bacteria, and tumor cells (*2–14*). IPCs express CD4 and major histocompatibility complex (MHC) class II, but lack hematopoietic-lineage markers (*2–14*). The nature of IPCs—whether they represent dendritic cells (*6*, *12*, *14*) or cells of a distinct lineage (*7*, *9*)—has been controversial. There is a progressive loss of $CD4^+$ T lymphocytes and functional IPCs during human immunodeficiency virus (HIV) infection (*15*, *16*). Preservation of IPCs is associated with protection from opportunistic infections, suggesting the importance of IPCs in the host defense (*16*).

A plasmacytoid cell type from human tonsils and blood that lacks lineage markers also expresses CD4 and MHC class II (*17–21*). These cells differentiate into type 2 dendritic cells (DC2s) when cultured with interleukin-3 (IL-3) and CD40 ligand (*19*, *21*). Unlike monocyte-derived type 1 dendritic cells (DC1s) that induced type 1 T helper cell (T_H1) differentiation, DC2s induced type 2 T helper cell (T_H2) differentiation (*21*). Here we investigated whether DC2 precursors (pDC2s) represent IPCs. Human peripheral blood cells were separated into the following populations (*19*, *21*): (i) monocytes (over 90% purity), obtained by centrifugation through 52% Percoll, then magnetic bead depletion of B, T, and natural killer (NK) cells; (ii) $CD4^+CD3^-CD11c^+$ immature DCs (99% purity) and (iii) $CD4^+CD3^-CD11c^-$ pDC2s (99% purity), obtained by magnetic bead depletion of B, T, NK cells, and monocytes, followed by fluorescence-activated cell sorting (FACS) (Fig. 1, A and B); (iv) pDC2-depleted blood mono-

[1]Saint Vincents Hospital and Medical Center, New York, NY 10011, and Long Island Jewish Medical Center–Albert Einstein College of Medicine, New Hyde Park, NY 11040, USA. [2]DNAX Research Institute, Palo Alto, CA 94304, USA. [3]The CW Post Campus of Long Island University, Brookville, NY 11548, USA. [4]University of Medicine and Dentistry, New Jersey Medical School, Newark, NJ 07103, USA.

*To whom correspondence should be addressed.

Table 1. Precursor DC2 cells are the natural IFN-producing cells. Cells (2×10^5) were cultured for 24 hours with HSV. Without HSV, IFN activity from different cell types was less than 12.5 U/ml (*23*). PBMC: total blood mononuclear cells; pDC2-dep: blood mononuclear cells positively selected for expressing CD3, CD11c, CD19, CD14, and CD56; pDC2-enrich: blood mononuclear cells that were depleted of cells expressing CD3, CD19, CD14, and CD56; pDC2: FACS-sorted $CD4^+CD11c^-lin^-$ cells; $CD11c^+$ DC: FACS-sorted $CD11c^+lin^-$ immature DCs; Mo: monocytes; DC1: monocyte-derived DCs after 6 days of culture with either granulocyte-macrophage colony-stimulating factor (GM-CSF) + IL-4 or GM-CSF + IL-4 + CD40 ligand (*21*); DC2: pDC2-derived DCs after 6 days of culture with IL-3 or IL-3 + CD40 ligand (*23*). ND, not determined.

	IFN (U/ml)							
	PBMC	pDC2-dep	pDC2-enrich	pDC2	$CD11c^+$DC	Mo	DC1	DC2
Exp. 1	500	ND	2,800	89,800	120	ND	<12.5	1,100
Exp. 2	40	<12.5	180	20,000	<12.5	350	<12.5	<12.5
Exp. 3	700	40	2,800	638,000	70	90	ND	ND

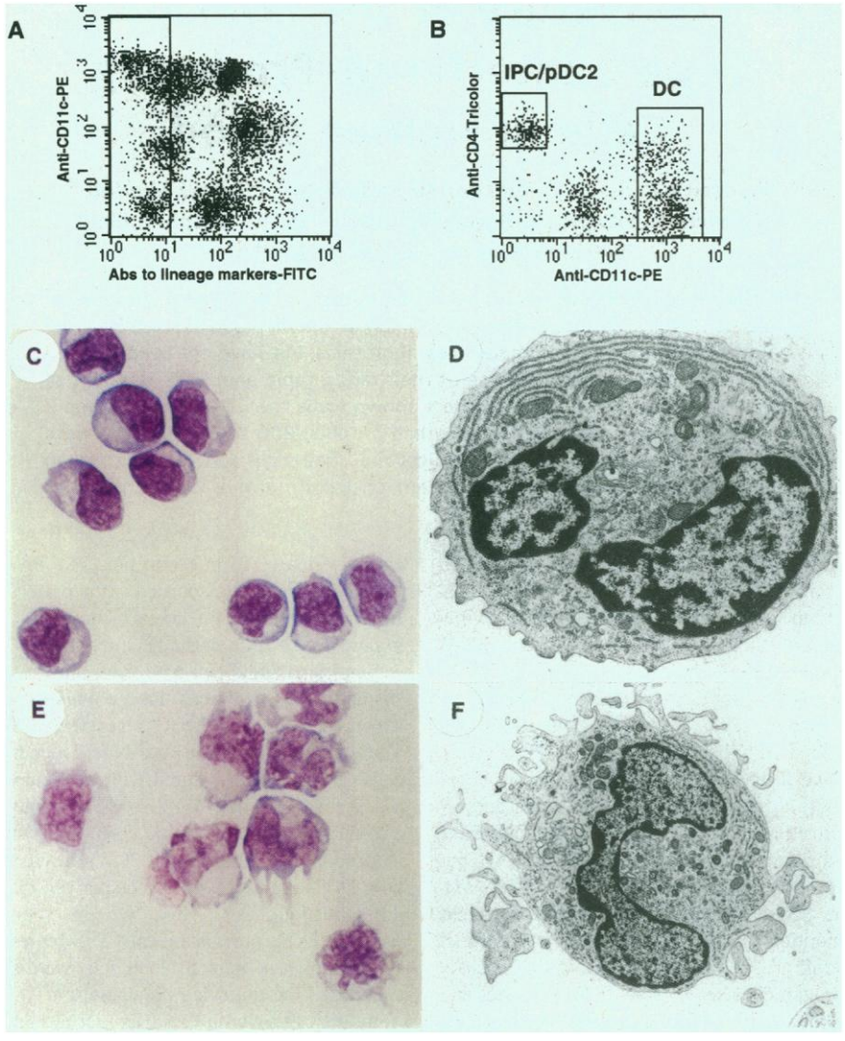

Fig. 1. Tracing and isolation of IPCs/pDC2s from human peripheral blood. $CD3^+$ T cells, $CD19^+$ B cells, $CD16^+$ and $CD56^+$ NK cells, and $CD14^+$ monocytes were depleted from blood mononuclear cells by immunomagnetic beads (Dynabeads M-450; Dynal, Oslo, Norway). The cells were stained with anti-CD4-Tricolor (Immunotech, Marseille, France), anti-CD11c-PE (Becton Dickinson, San Jose, California), and a mixture of fluorescein isothiocyanate–labeled antibodies to CD3, CD15, CD16, CD20, CD57 (Becton Dickinson), CD14 (Coulter, Miami, Florida), and CD34 (Immunotech). Within the lineage-negative population (**A**), $CD4^+CD11c^-$ IPCs and $CD11c^+$ immature DCs were isolated (**B**). IPCs are plasmacytoid by Giemsa staining (**C**) and contain rough endoplasmic reticulum and Golgi apparatus under transmission electron microscopy (**D**). The $CD11c^+$ blood immature DCs display dendrites (**E** and **F**).

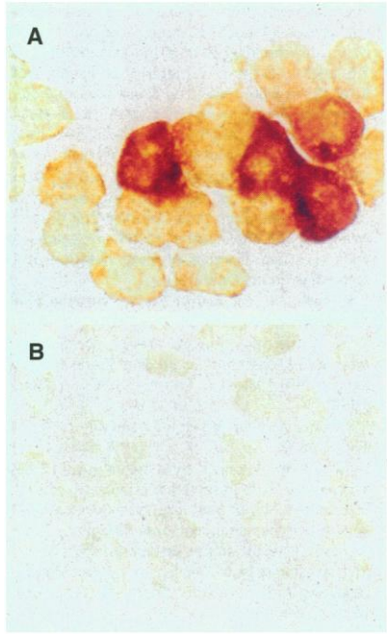

Fig. 2. Immunoperoxidase staining for IFN-α (*21*, *22*). (**A**) Purified $CD4^+CD11c^-lin^-$ IPCs were stimulated with HSV for 6 hours. (**B**) IPCs cultured in medium for 6 hours. The isotype controls for the primary antibody in both cell preparations show no staining.

nuclear cells; and (v) pDC2-enriched blood mononuclear cells (*19*). pDC2s have a plasmacytoid morphology, with rough endoplasmic reticulum and Golgi apparatus (Fig. 1, C and D). The $CD11c^+$ blood immature DCs display short dendrites (Fig. 1, E and F). The frequency of pDC2s in human blood mononuclear cells is less than 0.5% and increased to 3 to 10% after magnetic bead depletion of lineage-positive cells. The pDC2-depleted population contains B, T, NK cells, monocytes, and DCs. These cell populations were exposed to ultraviolet (UV)-irradiated herpes simplex virus (HSV) for 24 hours (*22*), and IFN in the culture supernatant was measured by a bioassay (*23*).

IFN production by total peripheral blood mononuclear cells (PBMCs) from three donors was 40, 500, and 700 international units (IU) per 2×10^5 cells (Table 1). There was a four to six times increase in IFN production from pDC2-enriched blood mononuclear cells (180 to 2800 IU per 10^5 cells) and a 180 to 911 times increase in IFN generation from purified pDC2s (20,000 to 638,000 IU per 10^5 cells). pDC2-depleted PBMCs, immature $CD11c^+$ DCs, monocytes, and monocyte-derived DC1s produced little or low levels of IFN. The ability of pDC2s to produce IFN was decreased after maturation into DC2s by culture with IL-3 or IL-3 plus CD40L for 6 days (Table 1). The geometric mean IFN-α generation by pDC2s was ~1 IU per cell, similar to previous estimates (*2*). Immunoperoxidase staining for human IFN-α confirmed that most pDC2s contained IFN-α protein after 6 hours of exposure to HSV (Fig. 2) (*22*). Analysis of IFN-α and IFN-β mRNA by polymerase chain reaction (PCR) showed that among human blood cells, pDC2s were making the most IFN-α and IFN-β mRNA (Fig. 3) (*24*). Thus, the blood cells responsible for IFN generation in response to HSV, previously known as the IPCs, are actually the DC2 precursors. These cells can be traced and isolated by their expression of CD4 or IL-3 receptor after depletion of cells expressing lineage markers and CD11c (*19*, *21*).

The purified IPCs also produced high levels of IFN in response to Sendai virus and heat-killed *Staphylococcus aureus*, confirming the previous studies on PBMCs and partially purified IPCs (*2–14*). The ability of UV-irradiated virus and heat-killed bacteria to induce IFN production by IPCs suggests that viral infection is not required for triggering IFN production. The rapid production of IFN by IPCs in the absence of other cells suggests that IPCs represent an effector cell type of the innate immune system. We propose that the IPCs/pDC2s should be included in the hematopoietic developmental chart as a distinct cell lineage. They function as professional IFN-producing cells at the precursor stages and as professional anti-

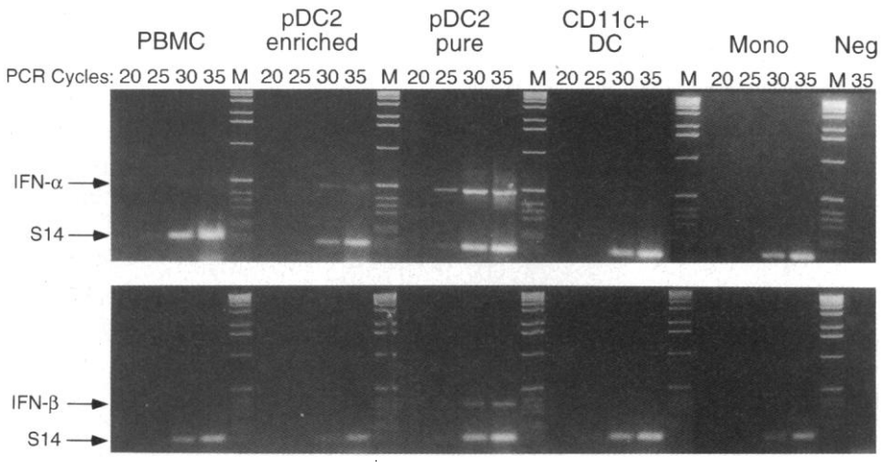

Fig. 3. RT-PCR amplification of IFN-α and IFN-β. PCR products amplified from each cell population after 20, 25, 30, or 35 cycles were separated on a 2% agarose gel containing ethidium bromide. Negative controls contained no cDNA. Marker is 1-kb DNA Ladder (Life Technologies, Grand Island, New York). IFN-α mRNA is apparent in PBMCs, is increased in the DC2 precursors (pDC2s) with enrichment and purification, and is diminished in the monocyte fraction. IFN-β mRNA is visualized only in the most highly purified DC2 precursors.

gen-presenting type 2 DCs upon terminal differentiation.

Type 1 IFNs have pleiotropic effects on the immune system, including up-regulation of MHC class I on all cell types and activation of macrophage and NK cells (*2*). IFNs are also critical in the activation and survival of both $CD4^+$ and $CD8^+$ T cells (*25*, *26*). Now with the ability to trace and isolate IPCs, it should be possible to directly study the interaction between IPCs and other cell types within the immune system. IFN-α has been widely used for treating hepatitis B and C as well as various cancers. A progressive loss of IPCs has been observed during HIV infection, suggesting that IPCs may represent targets for HIV-mediated infection and deletion. The present study provides an approach to directly monitor the number and functional state of IPCs in these patients. The ability to purify and culture IPCs in vitro will allow further studies on the molecular mechanisms that control the survival and growth of IPCs and their production of IFN, which may lead to novel therapies for patients with viral infections and cancer.

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22. Cells were incubated with UV-irradiated HSV in quadruplicate wells (2×10^5 cells in 200 μl of culture medium per well with 2×10^4 plaque-forming units of virus in 96-well culture plates) (*23*). IFN in supernatants from 24-hour cultures with and without IL-3 were analyzed with cytopathic reduction in human foreskin fibroblast monolayers cultured with vesicular stomatitis virus (sensitivity, 2 to 25 IU of IFN per milliliter) (*23*). Cytocentrifuge preparations of cells from 6-hour cultures were prepared for IFN-α immunostaining with mouse monoclonal antibody 7N4-1 (10 μg/ml; Schering-Plough Research Institute, Kenilworth, NJ) (*21*). Cells from 6-hour cultures were centrifuged and the sediment frozen for IFN-α and IFN-β mRNA PCR analyses (*24*).
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24. Reverse transcriptase (RT)–PCR: RNA was isolated with the acid guanidinium thiocyanate–phenol-chloroform method [P. Chomczrski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987)]. DNA contamination was removed by digestion with deoxyribonuclease I (5 U; Boehringer-Mannheim, Indianapolis, IN) for 30 min at 37°C. Controls without RT were performed. Reverse transcription was carried out with pd(T)12-18 (Pharmacia, Alameda, CA) priming and Superscript II RT (Life Technologies, Grand Island, NY). PCR reaction volume was 25 μl containing 100 ng of each primer, 40 nM of each deoxynucleoside triphosphate, 1 μl of cDNA, and 1.25 U of AmpliTaq (Perkin-Elmer, Foster City, CA). The primers used were as follows: IFN-α (sense: 5′-GATGGCCGTGCTGGTGCTCA-3′; antisense: 5′-TGATTTCTGCTCTGACAACCTCCC-3′; probe: 5′-CTCAAGCCATCTCTGTCCTCCATGAGATGA-3′); IFN-β (sense: 5′-TTGAATGGGAGGCTTGAATA-3′; antisense: 5′-CTATGGTCCAGGCACAGTGA-3′; probe: 5′-GGCTGGAATGAGACTATTGTTGAGAACCTC-3′); and human ribosomal protein S14 (sense: 5′-GGCAGACCGAGATGAATCCTCA-3′; antisense: 5′-CAGGTCCAGGGGTCTTGGTCC-3′). Each PCR amplification contained primers for the ribosomal protein S14, to verify the amounts of cDNA. A GeneAmp PCR System 9700 (Perkin-Elmer/Applied Biosystems) was used with an initial denaturation step of 94°C for 5 min, followed by cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 60 s, and a final elongation step of 72°C for 7 min. PCR products were separated on a 2% agarose gel, followed by DNA blotting and hybridization with ^{32}P-labeled probes.
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8 February 1999; accepted 3 May 1999

Math1: An Essential Gene for the Generation of Inner Ear Hair Cells

Nessan A. Bermingham,[1,2] Bassem A. Hassan,[1,2] Steven D. Price,[7] Melissa A. Vollrath,[5] Nissim Ben-Arie,[2]* Ruth Anne Eatock,[5,6] Hugo J. Bellen,[1,2,4,5] Anna Lysakowski,[7] Huda Y. Zoghbi[1,2,3,4,5]†

The mammalian inner ear contains the cochlea and vestibular organs, which are responsible for hearing and balance, respectively. The epithelia of these sensory organs contain hair cells that function as mechanoreceptors to transduce sound and head motion. The molecular mechanisms underlying hair cell development and differentiation are poorly understood. *Math1*, a mouse homolog of the *Drosophila* proneural gene *atonal*, is expressed in inner ear sensory epithelia. Embryonic *Math1*-null mice failed to generate cochlear and vestibular hair cells. This gene is thus required for the genesis of hair cells.

The inner ear initially forms as a thickening of the ectoderm, termed the otic placode, between rhombomeres 5 and 6 in the hindbrain. The otic placode gives rise to neurons of the VIIIth cranial nerve and invaginates to become the otocyst, from which the inner ear