Brief Definitive Report

REDUCED INTERFERON-GAMMA mRNA LEVELS IN HUMAN NEONATES

Evidence for an Intrinsic T Cell Deficiency Independent of Other Genes Involved in T Cell Activation

BY DAVID B. LEWIS,* ALF LARSEN,‡ AND CHRISTOPHER B. WILSON*

*From the *Department of Pediatrics, University of Washington and the *Division of Infectious Diseases, Children's Orthopedic Hospital and Medical Center, and the ‡Department of Molecular Biology, Immunex Corporation, Seattle, Washington 98105*

T cell activation by mitogens results in the accumulation of IL-2, IL-2-R, and IFN-γ mRNA, which are undetectable in resting T cells. Coordinate regulation of IL-2 and IFN-γ gene expression has been proposed based on similar kinetics of their mRNA accumulation in a human T cell line (1). However, in a recent report (2), peak transcription of these genes was temporally separated in activated peripheral blood T cells. We recently noted that IL-2 production and IL-2-R density of human neonatal T cells were comparable to adult cells, but IFN-γ production was markedly reduced (3). We now report the kinetics of mRNA accumulation for IFN-γ, IL-2, IL-2-R, and T3 delta chain (T3δ) in neonatal and adult mononuclear cells (MC) and T cells. We provide evidence for an intrinsic T cell deficiency in IFN-γ production in neonates at the pretransitional level, and separate modulation of IFN-γ and IL-2 gene expression.

Materials and Methods

Cell Preparations. MC were isolated from adult peripheral blood or umbilical cord blood from healthy term neonates on Ficoll-Hypaque density gradients as described (4). Purified T cells were prepared by treatment of MC with T cell Lymphokwik as specified by the manufacturer (One Lambda, Los Angeles, CA) followed by rosetting with 2-aminoethylisothiouronium–treated SRBC for 2 h at 4°C. The purity of the T cell preparations was assessed by indirect immunofluorescence using mAbs: 9.6-CD2, SRBC receptor; 64.1-CD3; 2H7-Bp32, B cells; 5F1-CDW14, monocytes; 1G10-CDW15, granulocytes; M21-IgG1 murine myeloma protein (Litton Bionetics, Kensington, MD); 9.6, 64.1, and 2H7 (Genetic Systems, Seattle, WA); and 5F1 and 1G10 were provided by Dr. I. Bernstein, Fred Hutchinson Cancer Research Center, Seattle, WA. All T cell preparations were ≥99% positive with 9.6 and 64.1 and were ≤2% positive with M21, 2H7, 5F1, and 1G10.

Induction and Assay of Lymphokines. MC or T cells were cultured and stimulated with 25 μg/ml Con A (Pharmacia Fine Chemicals, Piscataway, NY) and 50 ng/ml PMA (Sigma Chemical Co., St. Louis, MO), which induce maximal supernatant IFN-γ activity (3). After the indicated period of incubation, supernatants were collected, frozen at −70°C, and later assayed for IL-2 or IFN-γ (3, 4). The IL-2 activity of supernatants was determined by murine CTLL-2 (IL-2-dependent CTL line) cell proliferation (5) and the IFN-γ content

This work was supported by grants HD 18184, HD 06706, and AI 00568 from the National Institutes of Health, and by grant R-336-83 from the United Cerebral Palsy Foundation.

of the supernatants was determined by radioimmunoassay (Centocor, Inc., Malvern, PA) (3).

RNA Isolation and Northern Blots. Total RNA was isolated from MC or T cells by the guanidinium thiocyanate/cesium chloride method (6). RNA from a human B cell line, provided by Dr. Donald Pious, University of Washington, was used as a negative control. RNA samples were electrophoresed in 2.2 M formaldehyde, 1% agarose gels as described (6), and transferred either to nitrocellulose or to nylon membranes (Schleicher and Schuell Inc., Keene, NH) by capillary blotting. Filters were dried, baked for 1 h at 80°C, and the positions of 28 S and 18 S rRNA bands were determined by UV shadowing.

The following cDNA clones were subcloned into transcription vectors (Promega Biotec, Madison, WI) downstream from the SP6 promoter: (*a*) IFN-γ, nucleotides 266–860 of clone p52 (7); (*b*) IL-2, nucleotides 1–~700 (8) of IL-2 clone ASHuE × IL2; and (*c*) T3 delta chain clone, 3AIO, consisted of nucleotides 369–614 (9). Two IL-2-R clones were used: SP.3, nucleotides 68-376, and pBg1.2, nucleotides 376–611, both of which were described previously (10). The IL-2, IL-2-R, and T3δ clones were provided by Immunex Corporation, Seattle, WA, and the IFN-γ cDNA was provided by P. Gray, Genentech Corp., San Francisco, CA. [^{32}P]RNA probes were transcribed using SP6 polymerase and reaction conditions recommended by the manufacturer (Promega Biotec). Filters were hybridized with [^{32}P]RNA probes as previously described (10), then washed at 63°C in 6× SSC, 0.1% SDS for 30 min, followed by 0.1× SSC, 0.1% SDS for 30 min, before autoradiography at −80°C. In some cases, previously probed Nytran filters were stripped by boiling in 20 mM Tris pH 8.5, 2 mM EDTA, 0.1% SDS for 15 min, dried, and then reprobed.

Adult and neonatal cells were stimulated and processed in parallel in each experiment. mRNA levels were quantitated by densitometry and integration of autoradiograph bands. 100 U of activity was arbitrarily assigned to the 8 h adult value for each probe and experiment for the purpose of normalization. For IL-2-R mRNA densitometry, the 1.5-kb, 8-h adult band was used as the reference value both for the 3.5- and the 1.5-kb mRNAs.

Results

The kinetics of IFN-γ mRNA accumulation in Con A and PMA stimulated MC from three different pairs of adult and neonatal subjects were studied. Fig. 1 is a representative Northern blot from one of three experiments. Initially undetectable in adult MC, IFN-γ mRNA was measurable by 1 h, substantially increased by 4 h, peaked at ~8 h, and consistently declined by 16 to 24 h. In marked contrast, neonatal MC IFN-γ mRNA was barely detectable at 8 h and was undetectable at all other times, even after incubation for up to 96 h. IFN-γ was not detectable in unstimulated adult or neonatal MC.

To determine if the observed minimal accumulation of IFN-γ mRNA in neonatal MC was an intrinsic property of T lymphocytes, RNA of highly purified T cells from two adult and three neonatal subjects was analyzed (Fig. 2 and Table I). While both adult and neonatal T cells showed the highest IFN-γ mRNA accumulation after 8 h of incubation, followed by a consistent decline by 24 h, the peak level in neonatal cells was markedly lower than in adult cells. The peak amount of IFN-γ mRNA measured in the five subjects correlated well ($r = 0.99$) with the maximal amount of IFN-γ in supernatants (data not shown).

IL-2, IL-2-R, and T3δ mRNA were equally or more abundant in neonatal cells than in adult cells (Fig. 2, Table I), and the peak amount of IL-2 mRNA accumulated correlated well ($r = 0.98$) with supernatant IL-2 activity at 24 h of incubation (data not shown). The 3.5-kb IL-2-R mRNA was maximal at 8 h and declined by 24 h. The 1.5-kb species either declined less rapidly or actually

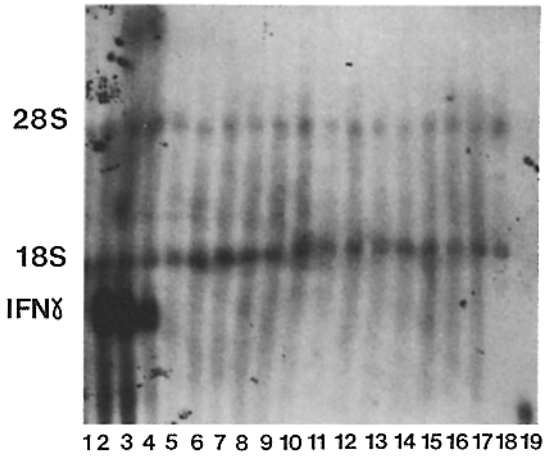

FIGURE 1. Total RNA from neonatal and adult mononuclear cells hybridized with IFN-γ probe. All lanes were loaded with 5 μg of total RNA. IFN-γ mRNA and 28 S and 18 S rRNA positions are indicated. Lanes *1–5*, adult MC incubated for 0, 4, 8, 24, and 48 h, respectively, with Con A and PMA. Lanes *6–12*, neonatal MC incubated for 0, 4, 8, 24, 48, 72, and 96 h, respectively, with Con A and PMA. Lanes *13–14*, unstimulated adult MC incubated for 24 and 48 h, respectively. Lanes *15–17*, unstimulated neonatal MC incubated for 24, 48, and 96 h, respectively. Lane *18*, B cell RNA. Lane *19*, ~200 bp of sense strand transcript of IFN-γ cDNA.

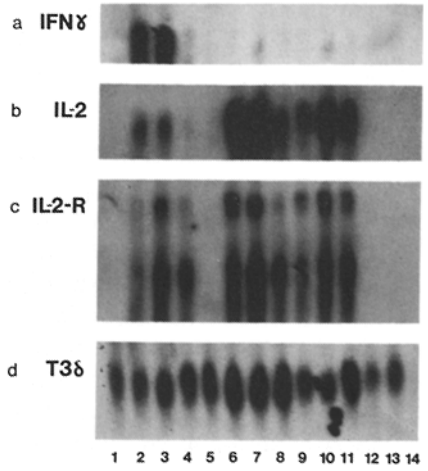

FIGURE 2. Total RNA from T cells of an adult and two neonatal subjects hybridized with IFN-γ, IL-2, IL-2-R (SP.3), and T3δ probes. All lanes were loaded with 5 μg of total RNA. Lanes *1–4*, adult 2 T cells incubated for 0, 4, 8, and 24 h, respectively, with Con A and PMA. Lanes *5–8*, neonate 2 T cells incubated for 1, 4, 8, and 24 h, respectively, with Con A and PMA. Lanes *9–11*, neonate 3 T cells incubated for 4, 8, and 24 h, respectively, with Con A and PMA. Lanes *12–13*, unstimulated adult 2 and neonate 2 T cells incubated for 8 h. Lane *14*, B cell RNA. Relative mRNA levels based on densitometry of these blots are presented in Table I, Exp. 2.

increased between 8 and 24 h both in adult and in neonatal T cells. Similar results were obtained with either the SP.3 (Fig. 2, Table I) or the pBg1.2 (data not shown) IL-2-R probes. T3δ mRNA, which was present in unstimulated cells,

TABLE I

Neonatal and Adult T Cell mRNA Levels of IL-2, IL-2-R, and T3δ

Exp.	mRNA levels of:	Subject used	Con A + PMA					Unstimulated
			0 h	1 h	4 h	8 h	24 h	8 h
1	IFN-γ	Neonate 1	0	ND	0.3	0.8	ND	0
		Adult 1	0	13.0	67.2	100	43.5	0
	IL-2	Neonate 1	0	ND	36	79	ND	0
		Adult 1	0	2	60	100	64	0
	IL-2-R* 1.5 kb	Neonate 1	0	ND	26	28	ND	0
		Adult 1	0	1	3	100	104	0
	IL-2-R 3.5 kb	Neonate 1	0	ND	56	29	ND	0
		Adult 1	0	1	12	56	17	0
	T3δ	Neonate 1	8	ND	109	53	ND	34
		Adult 1	3	17	28	100	17	14
2	IFN-γ	Neonate 2	ND	0	0	3.6	0	0
		Neonate 3	ND	ND	0	2.0	0.1	ND
		Adult 2	0	ND	91.5	100	1.4	0
	IL-2	Neonate 2	ND	0	2,115	9,711	552	0
		Neonate 3	ND	ND	385	1,394	846	ND
		Adult 2	0	ND	61	100	0	0
	IL-2-R 1.5 kb	Neonate 2	ND	0	43	266	189	0
		Neonate 3	ND	ND	101	115	189	ND
		Adult 2	0	ND	6	100	50	0
	IL-2-R 3.5 kb	Neonate 2	ND	0	84	100	14	0
		Neonate 3	ND	ND	61	90	36	ND
		Adult 2	0	ND	2	35	10	0
	T3δ	Neonate 2	ND	88	251	195	237	49
		Neonate 3	ND	ND	54	53	158	ND
		Adult 2	32	ND	46	100	97	25

Results are densitometric determinations from Northern blots as described in Materials and Methods.

* The SP.3 IL-2-R probe was used in the above experiments. Similar IL-2-R mRNA levels were obtained for all of the above adult and neonatal T cell samples when pBg1.2 probe was employed.

increased between 4 and 24 h after stimulation to similar peak amounts in neonatal and adult T cells.

Discussion

Using the Northern blot technique, we examined the kinetics of the accumulation of IFN-γ mRNA and compared it with mRNA levels of other genes expressed in activated neonatal and adult T cells. Both adult and neonatal MC or T cells had an approximate 8-h peak of IFN-γ mRNA. However, neonatal cells accumulated markedly less IFN-γ RNA than adult cells in all experiments. Neonatal T cell IL-2 mRNA accumulation was either comparable to or greater than that of adult T cells, and in both neonates and adults displayed kinetics similar to IFN-γ mRNA. The supernatant IFN-γ and IL-2 content of neonatal and adult T cells correlated well with their respective peak amounts of mRNA, suggesting that IFN-γ and IL-2 production were primarily regulated pretranslationally in both cell types. Neonatal and adult T cells had similar kinetics and amounts of T3δ chain and IL-2-R mRNA accumulation. Together, these results suggest that the reduced IFN-γ mRNA levels in neonates was selective and not found in other genes involved in T cell activation.

IFN-γ production appears to be partially dependent on the ability of IL-2 to bind to functional IL-2-R (11). Thus, reduced IFN-γ mRNA levels in neonatal compared with adult T cells could result from a greater proportion of internally

spliced IL-2-R mRNA, which does not appear to code for a functional protein (10, 12). However, we found similar IL-2-R mRNA levels in each of the adult and neonatal T cell samples using probes transcribed from either clone pBg1.2, in which 209 of the total 235 nucleotides are within the internally spliceable segment, or clone SP.3, in which 301 of the total 308 nucleotides are located 5′ to the internally spliceable segment. The pBg1.2 probe should detect only IL-2-R mRNA that has not been internally spliced, whereas the SP.3 probe should detect both. These results are consistent with recent studies in which we detected similar numbers of high and low affinity IL-2-R on adult and neonatal T cells after Con A stimulation (3). We noted using either probe that the 3.5-kb IL-2-R mRNA declined more rapidly than did the 1.5-kb species after 8–24 h incubation; this observation is of uncertain significance.

Our data suggest that reduced IFN-γ production is intrinsic to the neonatal T cell at the pretranslational level and not due to a qualitative macrophage defect as proposed by Taylor and Bryson (13). The similar kinetics of adult and neonatal IFN-γ mRNA accumulation suggest that there is no delay in neonatal signal transduction, but do not rule out a deficiency in signal amplification. Alternatively, decreased IFN-γ mRNA levels in neonatal T cells might be attributable to differences in transcription and RNA processing and stabilization, although major differences in processing were not detected by Northern blot analysis. An inhibitory or deficient T cell subset could explain the markedly reduced IFN-γ mRNA production observed in unfractionated T cells, although we have been unable to show monocyte- or lymphocyte-mediated suppression of neonatal MC IFN-γ production (3, 14). Further analysis will be needed to determine if our findings reflect a T cell subset or general T cell deficiency.

Based on their similar kinetics of mRNA accumulation in the Jurkat T cell leukemia line and homologies in their 5′ flanking regions (15), a common regulatory pathway has been proposed for the expression of IFN-γ and IL-2 gene products (1). Our findings are consistent with this hypothesis in that IFN-γ and IL-2 mRNA levels both peak in adult and neonatal T cells after ~8 h. However, if such a regulatory mechanism exists, the neonatal T cell is an example of how the magnitude of expression of the IFN-γ gene can be independently modulated from the IL-2 gene product.

Summary

IFN-γ mRNA levels in human neonatal blood mononuclear cells or highly purified T cells were markedly lower than those of adult cells after incubation with Con A and PMA. In contrast, IL-2, IL-2-R, and T3δ chain mRNA levels were kinetically and quantitatively similar in neonatal and adult T cells. The peak amount of IFN-γ and IL-2 mRNA correlated well with IFN-γ and IL-2 detected in supernatants of both neonatal and adult T cells. These results suggest that reduced IFN-γ mRNA levels in neonatal T cells is due to an intrinsic deficiency at the pretranslational level and indicate that the magnitude of IL-2 and IFN-γ gene expression can be independently modulated pretranslationally.

We would like to thank Dr. Patrick Gray of Genentech, San Francisco, CA who generously provided the human IFN-γ cDNA; Genetic Systems, Seattle, WA for providing mAbs;

Judith Westall for performing the supernatant IL-2 and IFN-γ assays; and Kae Pierce for secretarial assistance.

Received for publication 24 October 1985 and in revised form 10 December 1985.

References

1. Wiskocil, R., A. Weiss, J. Imboden, R. Kamin-Lewis, and J. Stobo. 1985. Activation of a human T cell line: a two-stimulus requirement in the pretranslational events involved in the coordinate expression interleukin 2 and γ-interferon genes. *J. Immunol.* 143:1599–1603.
2. Kronke, M., W. J. Leonard, J. M. Depper, and W. C. Greene. 1985. Sequential expression of genes involved in human T lymphocyte growth and differentiation. *J. Exp. Med.* 161:1593–1598.
3. Wilson, C. B., J. Westall, L. Johnston, D. B. Lewis, S. Dower, and A. Alpert. 1986. Decreased production of interferon gamma by human neonatal cells: intrinsic and regulatory deficiencies. *J. Clin. Invest.* 77:860
4. Wilson, C. B., and J. S. Remington. 1979. Effects of monocytes from human neonates on lymphocyte transformation. *Clin. Exp. Immunol.* 36:511–520.
5. Gillis, S., M. Ferm, W. Ou, and K. Smith. 1978. T-cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* 120:2027–2034.
6. Maniatis, T., E. F. Fristch, and J. Sambrook. 1982. Extraction, purification, and analysis of mRNA from eukaryotic cells. *In* Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
7. Gray, P. W., D. W. Leung, D. Pennica, E. Yelverton, R. Najarian, C. C. Simonsen, R. Derynck, P. J. Sherwood, D. M. Wallace, S. L. Berger, A. D. Levinson, and D. V. Goeddel. 1982. Expression of human immune interferon cDNA in *E. coli* and monkey cells. *Nature (Lond.).* 295:503–508.
8. Taniguchi, T., H. Matsui, T. Fujita, C. Takaoka, N. Kashima, R. Yoshimoto, and J. Hamuro. 1983. Structure and expression of a cloned cDNA for human interleukin-2. *Nature (Lond.).* 302:305–310.
9. Van der Elsen, P., B. A. Shepley, J. Borst, J. E. Coligan, A. F. Markham, S. Orkin, and C. Terhost. 1984. Isolation of cDNA clones encoding the 20K T3 glycoprotein of human T-cell receptor complex. *Nature (Lond.).* 312:413–418.
10. Cosman, D., D. P. Cerretti, A. Larsen, L. Park, C. March, S. Dower, S. Gillis, and D. Urdal. 1984. Cloning, sequence, and expression of human interleukin-2 receptor. *Nature (Lond.).* 312:768–771.
11. Reem, G. H., and Y. Ning-Hsing. 1984. Interleukin 2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. *Science (Wash. DC).* 225:429–430.
12. Leonard, W. J., J. M. Depper, G. R. Crabtree, S. Rudikoff, J. Pumphrey, R. J. Robb, M. Kronke, P. B. Svetlik, N. J. Peffer, T. A. Waldmann, and W. C. Greene. 1984. Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. *Nature (Lond.).* 311:626–631.
13. Taylor, S., and Y. J. Bryson. 1985. Impaired production of γ-interferon by newborn cells in vitro is due to a functionally immature macrophage. *J. Immunol.* 134:1493–1497.
14. Wilson, C. B., and J. E. Haas. 1984. Cellular defenses against *Toxoplasma gondii* in newborns. *J. Clin. Invest.* 73:1606–1616.
15. Fujita, T., C. Takaoka, H. Matsui, and T. Taniguchi. 1983. Structure of the human interleukin-2 gene. *Proc. Natl. Acad. Sci. USA.* 80:7437–7441.