INTERLEUKIN 3 (IL-3) INDUCES TRANSCRIPTION FROM NONREARRANGED T CELL RECEPTOR γ LOCI IN IL-3-DEPENDENT CELL LINES

By YACOB WEINSTEIN,* KAZUHIRO MORISHITA,‡
JOHN L. CLEVELAND,§ AND JAMES N. IHLE‡

*From the *Faculty of Health Sciences, Microbiology and Immunology Unit, Ben Gurion
University of Negev, Beer Sheva, Israel; the ‡St. Jude Children's Research Hospital,
Memphis, Tennessee 38101; and the §Laboratory of Viral Carcinogenesis,
National Cancer Institute, Frederick, Maryland 21701*

T cells are derived in the bone marrow from a pluripotential stem cell that also gives rise to committed progenitors for B cells and cells of the myeloid lineages (1–3). The progeny of stem cells that become committed to the T cell lineage are characterized by the expression and somatic rearrangement of the α, β, γ, and δ T cell receptor (TCR) genes (4, 5). During the ontogeny of T cells it is hypothesized that the γ and δ genes are the first receptor genes to undergo rearrangement and expression based on the observation that in 14-d fetal thymocytes and in the immature, Lyt-2⁻, and L3T4⁻ thymocytes only the γ genes are rearranged and expressed (4, 6–9). After γ and δ rearrangements, the β loci, and subsequently the α loci, are rearranged. The events that are required for the initiation of TCR gene expression and rearrangement and/or the commitment to T cell lineage differentiation are not known.

Over the past several years a variety of hematopoietic growth factors have been purified to homogeneity and their respective genes have been cloned. Among these growth factors, IL-3 is hypothesized to be active on early, multilineage stem cells and has been shown to support the proliferation of blast cell colonies that characteristically have high replating efficiencies and give rise to multiple hematopoietic lineages in vitro (10–12). From these studies, however, it has not been possible to determine whether IL-3 supports the self-renewal of pluripotential stem cells and/or whether IL-3 supports the proliferation of stem cells that have the ability to commit to the B or T cell lineages. A possible role for IL-3 in early T cell lineage commitment has been speculated based on its ability to induce the expression of the T cell associated markers, Thy-1 and 20 αSDH (11). More recently, a role for IL-3 in lymphoid differentiation has been suggested by the characterization of IL-3-dependent

cell lines that will differentiate to either B cells or T cells (13, 14). A variety of IL-3-dependent cell lines have been derived during the past several years from long-term bone marrow cultures or from retrovirus-induced leukemias (15-19). The phenotypic properties of these cell lines suggest that they are transformed with regard to their ability to terminally differentiate and appear to represent intermediates in early stem cell differentiation. For these reasons we have examined several of these lines for rearrangement and expression of the TCR genes. The results demonstrate that all the IL-3-dependent lines examined express the TCR-γ gene, that expression is from the nonrearranged loci, and that IL-3 regulates the expression of these genes.

Materials and Methods

Cell Lines and Growth Factors.   The leukemia cell lines were isolated from primary retrovirus-induced leukemias and maintained as described in detail (17, 18). IL-3 was obtained from WEHI 3-conditioned media and was purified to homogeneity (10, 20). Purified, homogeneous granulocyte (G)-CSF was kindly provided by N. Nicola (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia).

Northern Blot Hybridization.   Poly(A)$^+$ RNA was prepared by standard techniques (21) and samples of 5 μg of RNA were separated in 1% agarose gels in formaldehyde and blotted onto nitrocellulose. The filters were hybridized for 16 h at 42°C in 5× SSC containing 50% formamide and 2× Denhardt's solution with a ^{32}P probe prepared by random primer labeling. The filters were washed in 0.1× SSC at 55°C for 30 min.

Southern Blot Hybridization.   High molecular weight DNA was prepared by the standard techniques (21) from the cell lines and was restricted with Eco RI, electrophoresed in 0.8% agarose gels, and blotted onto nitrocellulose filters. The filters were hybridized as above but were washed at 65°C for 90 min.

Genomic and cDNA Libraries.   cDNA was made from 10 μg of poly(A) RNA using commercial reagents (Amersham Corp., Arlington Heights, IL). Eco RI linkers were used to clone into the λ gt10 (NFS-58 library) or λgt11 (FDC-P1 library). Genomic libraries were made from Eco RI partially digested DNA prepared from the NFS-60 cell line that was cloned into the phage EMBL-3 by the methods described by Maniatis et al. (21).

DNA Sequencing.   Nucleotide sequences were determined by the dideoxy chain termination method of Sanger et al. (22) after cloning specific restriction fragments into the phage vector M13mp18 and M13mp19. Commercial sequencing reagents were used (U. S. Biochemical Corp., Cleveland, OH).

S1 Nuclease Protection Assay.   The hybridization probe was made from primer extended M13 clone that was uniformly labeled by ^{32}P[dATP]. The probe was purified on alkaline, denaturing agarose gels and hybridized with poly(A)$^+$ RNA in 80% formamide at 52°C for 16 h and then was digested with S1 nuclease (100 U) for 1 h (23, 24). The protected fragments were analyzed on 5% acrylamide urea sequencing gels. The size of the protected fragments was determined by sequencing of the same fragment.

Hybridization Probes.   The probe for the γ4 gene was the Hind III-Eco RI fragment from the BS γ cDNA (nucleotide 582 to the polyadenylation site, Fig. 3). The probe for the γ1-3 genes was a Pst I fragment from the previously characterized pHDS4 plasmid (25). The δ constant region probe was a gift of M. Davis (Stanford University, Palo Alto, CA).

Miscellaneous Assays.   The levels of 20αSDH were determined by enzyme assays as described (26). Cell lines expressing more than 100 pmol/h/10^8 cells were considered positive. The expression of myeloperoxidase (MPO) was determined by Northern blot hybridization as described (27).

Results

The properties of the cell lines examined in these studies are summarized in Table I. The FDC-P1 line was isolated from long-term bone marrow cultures (15). The

TABLE I

The Origin and Phenotype Properties of Cell Lines

Cell line	IL-3 dependence	Thy-1	Mac-1	20αSDH	MPO	γ1-3	γ4	δ	β
FDC-P1	+	+	−	466	−	−	+	−	−
DA-1	+	−	+	280	ND	+	−	−	−
DA-3	+	+	+	375	+	+	−	−	−
NFS-58	+	+	+	9,450	+	+	−	−	−
NFS-60	+	+	−	21,000	+	+	+	−	−
NFS-107	+	−	+	1,680	ND	+	−	+	−
NFS-61	−	−	+	<100	ND	−	−	−	−
NFS-124	−	−	+	<100	ND	−	−	−	−
WEHI-3	−	+	+	<100	+	−	−	−	−

IL-3-dependent cell lines require the continuous presence of IL-3 for growth. Thy-1 and Mac-1 expression were determined by immunofluorescence on a FACS. The levels of 20αSDH were determined by enzyme assays as described (26); lines expressing >100 pmol/h/10^8 cells were considered positive. The expression of MPO was determined by Northern blot hybridization as described (27). The expression of the γ loci is based on the Northern blot data shown in Fig. 1.

DA and NFS lines were isolated from tumors induced by Moloney leukemia virus (17) or CasBrM leukemia virus, respectively (18). Both IL-3-dependent and factor-independent myeloid cell lines (NFS-61, NFS-124) were isolated in the later studies. The WEHI-3 cell line is a growth factor–independent cell line that was isolated from a BALB/c myeloid leukemia (20). All the IL-3-dependent lines expressed 20αSDH. The lines variably expressed the lymphoid and myeloid related markers Thy-1 and Mac-1. None of the lines expressed L3T4, Lyt-2, or T3 or proliferated in response to IL-2 (data not shown). A number of the IL-3-dependent lines expressed the myeloid lineage marker myeloperoxidase (MPO). Morphologically, the lines were lymphoblastic to myeloblastic in appearance.

The cell lines were initially examined for the expression of TcR γ chain transcripts by Northern analysis using a probe that detected the γ4 locus or a probe for the γ1-3 loci (Fig. 1, *A* and *B*, respectively). As shown in Fig. 1 and summarized in Table I, all of the IL-3-dependent cell lines examined expressed transcripts for the γ1-3 loci and/or the γ4 locus. In all cases the major transcript was ~1.5 kb, similar to the predominant transcript detected in day 14 fetal thymocytes or in immature adult thymocytes (6). Most of the lines expressed RNAs that hybridized with the γ1-3 probe, while the NFS-60 cells contained transcripts detectable with both probes. The FDC-P1 cells only contained transcripts from the γ4 locus. In contrast, none of the IL-3-independent lines examined contained transcripts that hybridized with the γ probes. None of the cell lines contained detectable transcripts that hybridized with probes for the TCR-β gene, while one of the cell lines (NFS-107) expressed a 1.4-kb transcript detectable with a probe against the δ locus (data not shown).

We next examined the lines for rearrangements of the TCR genes by Southern blot analysis. As shown in Fig. 2, most of the cell lines contained no apparent rearrangements using a probe that detects γ1-3. The differences that are observed with the FDC-P1, NFS-58, and NFS-60 cell lines are due to genetic polymorphisms in
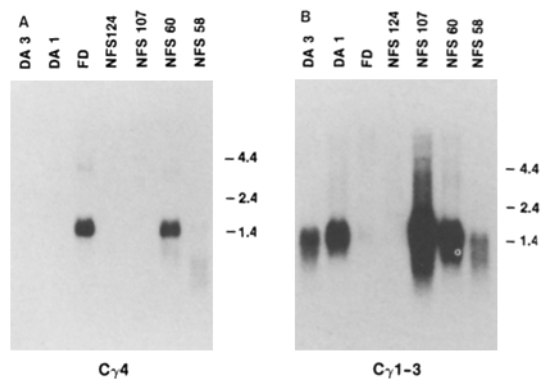
C γ4   C γ1-3

FIGURE 1.   Expression of γ transcripts in IL-3-dependent cell lines. Poly(A)$^+$ RNA was prepared by standard techniques (21) and 5 μg of RNA was separated in a 1% agarose gel in formaldehyde and blotted onto nitrocellulose. The filters were hybridized for 16 h at 42°C in 5× SSC containing 50% formamide and 2× Denhardt's solution with a ^{32}P probe prepared by random primer labeling. The filters were washed in 0.1× SSC at 50°C for 30 min. (A) The probe for the γ4 gene was the Hind III–EcoRI fragment from the BS γ cDNA (nucleotide 582 to the polyadenylation site, Fig. 4). (B) The probe for the γ1-3 genes was a Pst I fragment from the previously characterized pHDS4 plasmid (22).

the γ loci. The FDC-P1 cells are of DBA/2 origin and this strain lacks the γ3 pseudogene and contains a restriction enzyme polymorphism in the γ1 locus (15, 28). The NFS-58 and NFS-60 cell lines were derived from (NFS × DBA/2) F₁ mice and are therefore heterozygous for the presence or absence of the γ3 pseudogene and for the restriction enzyme polymorphism in the γ1 locus. Among the lines shown in Fig. 2, only the DA-2 cell line contained rearrangements of both alleles of the γ1 locus. DA-2 cells are growth factor–independent T cells that were included as a control and have a rearranged β locus, express both γ and β transcripts, and express
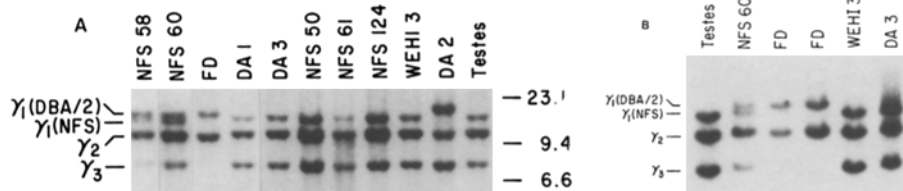

FIGURE 2.   Lack of detectable rearrangements of the γ1-3 loci in IL-3-dependent cell lines. (A) DNA from the indicated cell lines was restricted with Eco RI, electrophoresed in 0.8% agarose gels, and blotted onto nitrocellulose filters. The filters were hybridized as in Fig. 1 with the pHDS4 probe for the JCγ1 gene, which detected the γ1-3 loci. The relative mobilities of fragments of Hind III cut λ DNA are shown as standards. The cell lines, their properties, and their strain of origin are: NFS-58 and NFS-60, IL-3-dependent myeloid lines from (NFS × DBA/2)F₁ mice; FD, an IL-3-dependent myeloid line from a DBA/2 mouse; DA-1 and DA-3, IL-3-dependent myeloid lines from BALB/c mice; NFS-50, an IL-3-independent myeloid line from a (NFS × DBA/2)F₁ mouse; NFS-61, an IL-3-independent myeloid line from a (NFS × NFS.C58-C6 = 58v-1)F₁ mouse; NFS-124, an IL-3-dependent myeloid cell line from a (NFS.C57BR-H-2^k × NFS.AKR-Akv-1)F₁ mouse; WEHI-3, an IL-3-independent myeloid line from a WEHI mouse; DA-2, a T cell lymphoma cell line from a BALB/c mouse. Testes DNA was obtained from BALB/c mice. The lower hybridizing band that is absent in FD (DBA/2) cells is the γ3 pseudogene. The top doublet band seen in NFS-58 and NFS-60 represents the two γ1 alleles; the larger is derived from the DBA/2 parent and the lower band of the doublet is characteristic of the NFS parent, which is also found in BALB/c mice. Among the lines shown, only the FD cells would be expected to be homozygous for the upper band; the NFS-58, NS-60, and NFS-50 lines would be expected to be heterozygous and the remainder of the lines would be expected to be homozygous for the lower band. (B) Some of the above samples were electrophoresised as above for longer periods of time to allow for better resolution of the restriction fragments.

T3. None of the IL-3-dependent cell lines contained detectable rearrangements of the γ4 locus, as shown by Southern blot analysis with the γ4 probe (data not shown). Finally, none of the IL-3-dependent cell lines contained detectable rearrangements of the β locus (data not shown).

Because of the presence of γ transcripts without apparent rearrangements, we isolated cDNA clones from the FDC-P1 and NFS-58 cell lines. Two cDNA clones of 1.25 and 1 kb were isolated from the cDNA library made with FDC-P1 RNA. Both clones contained a polyadenylation site and the 1-kb clone had a sequence identical to the 1.25-kb clone, but lacked 5' sequences found in the longer clone. The sequence of the 1.25-kb clone is shown in Fig. 3. The sequences starting at position 197 and position 260 are identical to the published sequences for the Jγ4 and Cγ4 throughout the published sequences that end at position 1160 (28). The sequence from nucleotide 1–196, however, showed no homology to any of the published Vγ genes, suggesting that it either represented a unique V gene or possibly was derived from sequences 5' of the Jγ4 gene. Examination of the sequence shows that there are no open reading frames and the sequence would not encode a V region with the typical cysteine residues (4). The sequence showed homology, however, to sequences that are immediately 5' of the Jγ1 gene and contained the conserved nanomer/heptamer sequence separated by 12 nucleotides that is characteristically found immediately 5' of J genes and is involved in recombination. To determine whether the 5' sequences were derived from the region immediately 5' to the Jγ4 gene, this genomic region

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GGTCTGTGGATGCTTTCCAGTCATCACTACTTGGAGAAGAGAAGAGTGTG    50

TGAAGGAAACTTACAAACTATTGGATGATACCAAACTTTGCAAAGGAATC    100

CAGGTCTCTTTCTAAGATAAGAGTTCCTAAATGACTCATCATACTGAAGC    150
                    -     9   -     12    -   7  -J
TCAAGAGTGAGCTCC TGTTTTTGT AGAAGCGCTGAA CAATGTGT CAGGCA    200

CATCATGGGTCAAGATATTTGCCAAAGGGACAAAGCTCGTAGTAATTCCC    250
        JC
CCAGACAAACGCACTGACTCAGACTTTTCTCCCAAGCCTACTATTTTCCT    300

TCCTTCTGCTGCTGAAACAAATCTCCATAAGGCTGGGACCTACCTTTGTC    350

TCCTTGAAAAGTTCTTTCCTAAAGTCATAAGGGTGTATTGGAAAGAAAAG    400

GATGGTGAAAAGATTCTGGAATCCCAGGAAGGAAACACCATAAAAACTAA    450

TGACAGATACATGAAATTTAGTTGGCTGACGGTGACTGAAGATTCAATGG    500

CTAAAGAACATAGTTGCATTGTCAAACACGAAAACAACAAAAGAGGAGTT    550

GACCAAGAGATTCTGTTTCCTCCCATAGGTAAAGCTTTCACAACTATTAA    600

TGTTAATCCCAGAGATAGTGTTTTGCGGCATGAAAATGTTAACAATGCTA    650

CTGATCTGGAAGATTGCATGAAAGGAAGAAAGGATATGTTGCAGCTTCAG    700

GTCACCACCACCTATGCATTCTACACCTACCTCATCCTGTTCTTCAAGAG    750

CATGGTCCACTTGGCCTTCGTTGTATTCTGTCTATTCAGAAGAGCAGCCA    800
                              ***
TGTCCTGTGATGATCAACGTTCATAATGGATGGTGAAACAGGAGAGTCCT    850

GCTATCTTCATCCTTCCTGCATCTCAATATCTGCTGAGGGTCTAGCGGGG    900

CTTGATTGCTGTCTTTGACCTCCTCGAGGACACATATGCATGATTTTTGT    950

ATCATTACTATAGTTCTCACAGCAGAGTGGAAACTGATCAACTTTACTCT    1000

TCAACAGACCGTAGCTTCCAAGCATCCAGCCATCCTAACCCATCCTCTCT    1050

CAGCCCCTCTAGCAGATCCCAGCATCACAGGCCCAATGCACACAAACTGC    1100

TGCTCCTAGGGACCCCATTCATACCCTAGAACCAGATAATTCCCCCAGAG    1150

CCTCCACTTCATATGCCCTAAATAACTTTATTTGAACTTGGATATTGAGC    1200
                  ------
TTTGCTTTACAAAATACAGTAATAAAATCCAGACTACTTTTGGAAAAAAA    1250·
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FIGURE 3. Sequence of a γ cDNA clone isolated from cDNA libraries of FDC-P1 cells. The complete sequence of the BS γ cDNA clone isolated from FDC-P1 cells is given. The clone was obtained from a cDNA library prepared from total poly(A)⁺ RNA from FDC-P1 cells in λgt11. The Eco RI insert was subcloned into M13 mp8 and mp9 and sequenced by the dideoxy method using commercial sequencing reagents. The nanomer/heptamer sequence and the 12-base spacer are indicated. The beginning of the Jγ and Cγ regions are shown as are the translational termination signal (*) and the polyadenylation signal (-).

was cloned and sequenced and the sequence was found to be identical to that shown in Fig. 3 *A* (data not shown).

Three cDNA clones were isolated from libraries of the NFS-58 cell line. By restriction mapping, the clones were overlapping and therefore one clone was chosen for sequencing. The clone contained sequences that were identical to the published sequence of Jγ2 (29). The sequence of the clone immediately 5' of Jγ2 contained the nanomer/heptamer sequences and was identical to the published genomic sequence 5' of the Jγ2 segment. Therefore, in both the NFS-58 and FDC-P1 cells, transcription occurs from a nonrearranged locus and initiates at least 200 nucleotides 5' of the their Jγ loci.

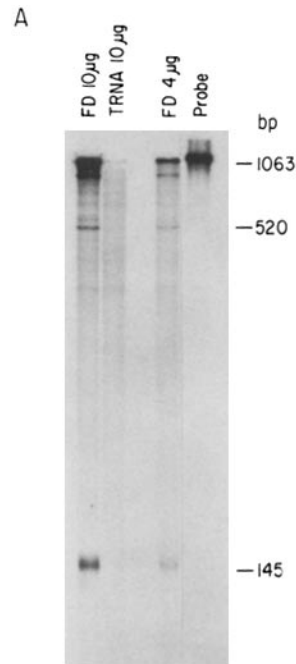Figure 4.   (*A*) S1 mapping of γ4 transcripts in FDC-P1 cells. Poly-(A)+ RNA was hybridized with uniformly labeled 1.1-kb, single-stranded DNA probe in 80% formamide at 52°C as explained in details in the Materials and Methods section. After 16 h the mixture was treated with S1 nuclease (100 U) and the protected fragments were analyzed on 5% sequencing gel. (*B*) Diagrammatic representation of the V1.2 J4 and Cγ4 loci. The black bar represents the Sac I–Hind III 1.1-kb fragment that was used as the probe. The sizes of the protected fragments are shown. The restriction sites for Hind III (H) and Sac I (S) are indicated. The restriction map is not necessarily complete. The position of the V and C exons is approximate and based on the restriction maps published by Hayday et al. (29) and Iwamoto et al. (28) and our own data.
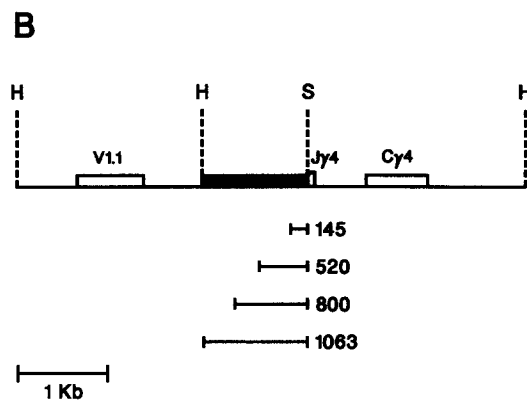
To initially determine the possible site of initiation of the transcripts in FDC-P1 cells, Northern blots were probed with fragments 5' to the J region. Hybridization was not detected with probes from the V region, while hybridization was seen with a 1.1-kb probe obtained from the region immediately 5' of the J sequences (data not shown). To more precisely determine the RNA structure, this probe was used in S1 protection experiments. The structure of the genomic fragment used for the protection experiments and the results are shown in Fig. 4. Four major protected fragments were seen. The largest protected fragment was the length of the probe indicating that some transcripts initiated 5' to the genomic fragment. Other protected fragments of ~800, 520, and 145 bp were seen. Since the predominant transcript in FDC-P1 cells is 1.3–1.4 kb, the majority of the transcripts contain sequences that start 174 bp 5' of the J. The presence of multiple protected fragments could indicate multiple transcriptional start sites or may be due to splicing of transcripts that initiate further 5' of the fragment.

The expression of the γ loci in IL-3-dependent cell lines, and not in any of the IL-3-dependent cell lines, suggested the possibility that IL-3 may be important for its expression. To examine this, exponentially growing FDC-P1 cells were removed from IL-3 and at various times the levels of γ4 transcripts were determined by Northern analysis. Throughout the times examined the viability of the cells was >95%. As shown in Fig. 5 A, removal of IL-3 resulted in a rapid decrease in the levels of γ4 transcripts such that by 4 h few or no transcripts were detectable. During this time there was no effect of the removal of IL-3 on the levels of β actin transcripts. We next examined the effects of readdition of IL-3. As shown in Fig. 5 B, when cells were deprived of IL-3 for 10 h and were stimulated with IL-3, there was a rapid reappearance of γ transcripts. The peak levels were observed at ~2 h after the addition of IL-3.
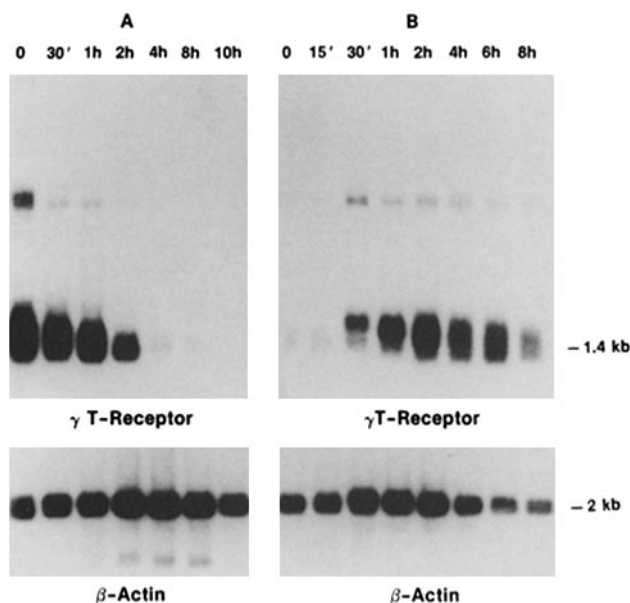

FIGURE 5. IL-3 regulation of γ transcripts in FDC-P1 cells. (A) Exponentially growing cells in IL-3 were harvested by centrifugation, washed twice, and resuspended in IL-3-free media. At the indicated times a portion of the cells was removed, total poly(A)$^+$ RNA was isolated as above, and it was analyzed by Northern blot analysis as in Fig. 1. (B) IL-3 (100 U/ml) was added to cells that had been grown in the absence of IL-3 for 10 h. At the indicated times, cells were collected, poly(A)$^+$ RNA was isolated, and it was analyzed for the expression of γ4 transcripts and for β actin transcripts as described in Fig. 1.

The affects of IL-3 on the levels of transcripts from γ loci in NFS-60 cells are shown in Fig. 6. When cells were removed from IL-3, the levels of transcripts detectable with a γ1-3 probe decreased with kinetics comparable to that seen in FDC-P1 cells. No transcripts were detectable in these experiments with probes for the γ4 locus. With the readdition of IL-3 transcripts detectable with probes for both the γ1-3 and γ4 loci reappeared with kinetics comparable to those seen with FDC-P1 cells. Since NFS-60 cells proliferate in response to G-CSF at a rate comparable to
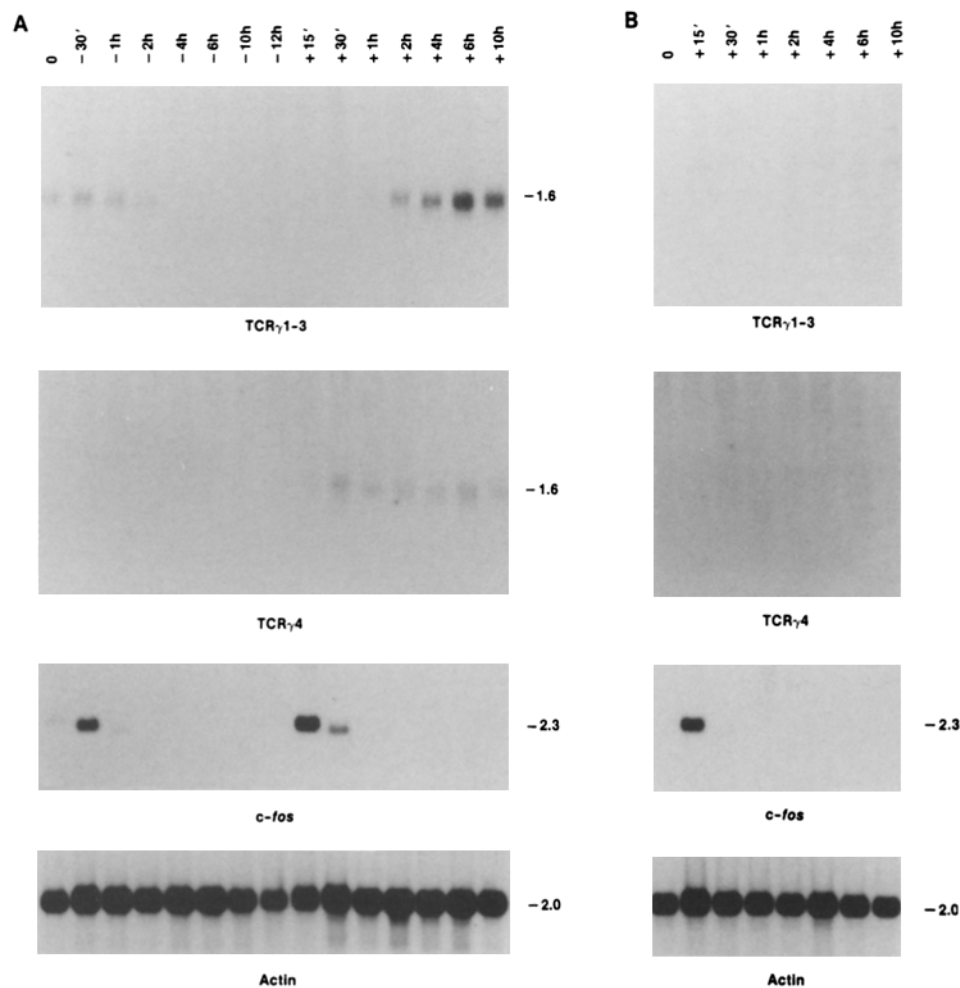

FIGURE 6.   IL-3 regulation of γ transcripts in NFS-60. (A) Exponentially growing cells in IL-3 were harvested by centrifugation, washed twice, and resuspended in IL-3-free media. At the indicated (-) times a portion of the cells was removed, total poly(A)$^+$ RNA was isolated as above, and it was analyzed by Northern blot analysis as in Fig. 1. At the indicated (+) times IL-3 (100 U/ml) (Fig. 4 A) or G-CSF (100 U/ml) (Fig. 4 B) was added to cells that had been grown in the absence of IL-3 for 10 h. Cells were collected, poly(A)$^+$ RNA was isolated, and it was analyzed for the expression of γ4, γ1-3, β actin, and c-fos transcripts as described in Fig. 1. Throughout the times examined the viability of the cells was >95%.

IL-3, we also examined the effects of the readdition of G-CSF on γ loci expression. As shown in Fig. 6 *B*, G-CSF did not induce detectable levels of transcripts that hybridized with probes for either γ1-3 or γ4. As a control the blots were re-hybridized with a c-*fos* probe. As shown in Fig. 6, both IL-3 and G-CSF induced a comparable but transient expression of c-*fos* transcripts.

Discussion

Our results demonstrate that nonrearranged TCR-γ loci are transcribed in IL-3-dependent myeloid leukemia cell lines. The expression of nonrearranged TCR γ loci in pre-T and pre-B cells isolated from Abelson-transformed thymocytes has been recently reported (30). These authors also detected expression of the TCR-γ locus in one myeloid cell line (FDC-P1), but either low (WEHI 3BD⁺) or no (W274) transcripts in other myeloid lines. Among the cell lines we have examined, all (6/6) of the IL-3-dependent lines expressed one or more of the TCR-γ loci, while none (0/3) of the IL-3-independent myeloid cell lines had detectable transcripts. The basis for the unique expression of TCR-γ loci in the IL-3-dependent cell lines is not known. It is possible that the factor-dependent and -independent cell lines represent different stages of differentiation. However, there were no consistent differences in the phenotypic markers examined, with the exception 20αSDH expression. Alternatively, the factor-independent cells, like the IL-3-dependent cells, may require IL-3 for TCR-γ expression. Unfortunately, this possibility cannot be examined since none of the IL-3-independent lines have detectable receptors for IL-3 (our unpublished data).

Whether all IL-3-dependent cell lines would express TCR-γ loci is not known. In particular, IL-3-dependent cell lines have been speculated to represent a variety of stages of hematopoietic stem cell differentiation and lineage commitment. For example, pro-B and pro-T IL-3-dependent cell lines have been described (13, 14) and it will be of interest to determine whether they might differ in TCR γ expression. Similarly, IL-3-dependent mast cell lines have been isolated (31), but it is not known whether these cells contain transcripts for TCR-γ loci comparable to those described above.

Although all of the IL-3-dependent cell lines had transcripts for one or more of the TCR-γ loci, only one of the cell lines contained transcripts for the TCR-δ locus. In addition we have found that several cell lines variably express transcripts that include the TCR-γ V region (data not shown). None of the cell lines examined had detectable transcripts for the TCR-β. Among the cell lines examined there have been not any apparent correlations between the expression of one or another of the TCR loci with the phenotypic markers examined. It should be noted that studies with fetal thymocytes have indicated that the TCR-γ loci may be the first TCR loci expressed during T cell differentiation (4).

The transcriptional initiation sites of the transcripts from the TCR-γ loci are not known. Sequencing of cDNA clones demonstrated that the transcripts contain sequences immediately 5' of the J region, thus indicating that transcription initiates further 5'. S1 protection experiments, with a probe from the region 5' of the J region, also indicated that the transcripts contained sequences immediately upstream of the J region. Assuming that the major 1.4-kb transcript terminates at the normal transcriptional termination site of the constant region, the size of the transcript would suggest that transcription initiates ~200 bp 5' of the J region. Consistent with this

hypothesis there was a major protected fragment in the S1 experiments that indicated a potential site for transcriptional initiation at 174 bp 5′ of the J region. Alternatively, transcription may initiate further upstream and the major 1.4 kb transcript may be produced by splicing to the site defined in the S1 protection experiments.

The results also demonstrate the unexpected result that TCR-γ transcripts were dependent on IL-3. The kinetics of the loss of transcripts in the absence of IL-3 and the reappearance of transcripts with the addition of IL-3 were very similar to those previously shown for c-*myc* transcripts in these cells after the removal or readdition of IL-3 (32). A specificity for IL-3 was demonstrated by the observation that although NFS-60 cells proliferate equally well in either IL-3 or G-CSF, TCR-γ transcripts were only detectable in cells stimulated with IL-3. This specificity for IL-3 relative to G-CSF could not be examined in the other cell lines since only NFS-60 cells have receptors for G-CSF. Several of the cell lines respond to other hematopoietic growth factors, including granulocyte macrophage (GM)-CSF, IL-4, and IL-6 and it will be of particular interest to determine which of these factors can induce γ locus transcription. In this regard, preliminary data indicate that DA-3 cells or NFS-107 cells proliferating in GM-CSF and IL-4, respectively, contain TCR-γ transcripts.

With regard to the cell lines that we have examined, it has not been possible to precisely define the stages of hematopoietic stem cell differentiation that they might represent based on their phenotypic markers. Since many of the cell lines were isolated from myeloid leukemias, transformation may contribute to this ambiguity. In contrast, the FDC-P1 cell line was isolated from long-term bone marrow cultures and has been speculated to have the properties of a normal early hematopoietic progenitor (15). However, whether these cells are able to differentiate along the lymphoid lineages either in vitro or in vivo is not known.

The significance of IL-3-regulated expression of nonrearranged TCR-γ loci is not known. In B cells, expression from nonrearranged Ig loci has been shown to be a prerequisite for recombination (33–35). Moreover, the ability of growth factors (IL-4) or mitogens to induce expression may affect recombination (36) in Ig class switching. Based on these observations it has been hypothesized that expression of nonrearranged Ig and TCR loci during development is important for recombination to occur. If IL-3 can regulate the expression of the nonrearranged TCR-γ loci in normal hematopoietic progenitors comparable to the regulation seen in the IL-3-dependent cell lines, it can be hypothesized that stem cells differentiating in the presence of IL-3 may be more likely to undergo TCR rearrangements than stem cells differentiating in other hematopoietic growth factors such as G-CSF.

Summary

The expression of the murine TCR-γ genes was examined in a series of IL-3-dependent and growth factor–independent cell lines. All of the IL-3-dependent cell lines, but none of the IL-3-independent lines, expressed high levels of one or more of the γ genes but did not express the TCR-β genes. None of the cell lines expressing the γ loci contained detectable genomic γ gene rearrangements. Sequencing of cDNA clones from two of the cell lines demonstrated that transcription was from nonrearranged γ loci based on the presence of sequences in the cDNAs that are found im-

mediately 5' of the Jγ4 and Jγ2 genes. The expression of γ transcripts was dependent upon IL-3 and no transcripts were detectable within 6–8 h after the removal of IL-3. Readdition of IL-3, but not granulocyte CSF, resulted in the reappearance of γ transcripts within 30 min. The results demonstrate that IL-3 regulates the expression of nonrearranged γ loci. Since expression is required for rearrangement, it can be hypothesized that IL-3 may influence the ability of lymphoid/myeloid progenitors to commit to the T cell lineage.

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