ONCOGENE EXPRESSION IN AUTOIMMUNE AND NORMAL PERIPHERAL BLOOD MONONUCLEAR CELLS

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by excessive B lymphocyte proliferation (1) and the spontaneous production of autoantibodies reactive with a variety of self antigens (1). Studies (2, 3) involving the families of patients with SLE have shown that genetic factors play a role in the development of this disorder. We have recently (4, 5) reported that a characteristic of lupus-like diseases in mice is the abnormal expression of specific cellular oncogenes (protooncogenes). Our group has shown (5) that B lymphocytes from NZB and BXSB mice express increased quantities of the c-myc and N-ras protooncogenes. In light of the abnormalities in protooncogene expression in autoimmune mice and the excessive activation of lymphocytes present in humans with systemic autoimmune diseases (6, 7), we investigated cellular oncogene expression in such patients.

Cellular oncogenes are DNA sequences homologous to the dominant growth-transforming elements of certain RNA tumor viruses (8). In normal cells they appear to be involved in the regulation of growth, activation, and differentiation (9–11). The proteins encoded by one group of protooncogenes (myc, myb, and fos) are found in the nucleus, and are believed to modulate gene expression, whereas those encoded by other cellular oncogenes (e.g. the ras family) reside at the cell surface, where they apparently function in the generation or transduction of growth factor signals (8, 12–15).

In this study we report that the expression of selected protooncogenes by PBMC of patients with SLE differs significantly from that of PBMC derived from normal donors. These differences were assessed by analyzing protooncogene expression by cells from patients with nonlupus autoimmune diseases and by mitogen activated normal lymphocytes.

Materials and Methods

Source and Preparation of Cells. All patients were under treatment at the Clinical Center of the National Institutes of Health, Bethesda, MD (Arthritis and Rheumatism Branch). The technique of apheresis was used to obtain $3–5 \times 10^9$ PBMC from patients and normal volunteers after written informed consent. All lupus patients met ARA criteria for SLE (16). Four of these patients had very active disease as defined by major clinical

TABLE I

Clinical and Laboratory Evaluation of Patients with SLE

Patient	Disease activity	Clinical symptoms	Laboratory evaluation*			
			C	Anti-DNA	ESR	WBC
1	Very active	Rash, psychiatric, myalgias, arthralgias	↓	↑	0	↓
2	Very active	Pleuritis, active glomerulonephritis	0	0	↑	↓
3	Very active	Pericarditis, active glomerulonephritis	↓	↑	↑	↓
4	Very active	Rash, psychiatric, arthralgias	0	ND	↑	↓
5	Active	Active glomerulonephritis	0	0	↑	0
6	Active	Rash and fevers	0	↑	ND	↓
7	Active	Myalgias and arthritis	↓	↑	↑	↓
8	Active	Rash, pleuritis	↓	↑	↑	↓
9	Active	Pleuritis and abdominal inflammation	↓	↑	0	↓
10	Active	Myalgias and arthralgias	0	↑	↑	↓
11	Active	Arthralgias, pleuritis	↓	0	↑	↓
12	Active	Mild glomerulonephritis	0	↑	↑	↓
13	Inactive	Mild rash	0	0	0	↓
14	Inactive	None	↓	↓	↑	↓
15	Inactive	None	0	0	↑	0
16	Inactive	None	0	0	0	↓
17	Inactive	None	↓	↑	0	0

Laboratory evaluation was done at the same time that blood samples were obtained for RNA analysis. Several patients did not have all of the laboratory studies performed at this time.

* C, hemolytic complement; anti-DNA, antibodies reactive with native DNA as measured by the Farr assay; ESR, erythrocyte sedimentation rate measured by the Westergren method and corrected for sex and hematocrit; WBC, peripheral leukocyte count; ↑, elevated compared with age- and sex-matched normals with $p < 0.05$, except for anti-DNA, where $p < 0.01$; ↓, reduced compared with age- and sex-matched normals, $p < 0.05$; 0, normal range; ND, not determined.

involvement of at least two organ systems in association with expected laboratory abnormalities. Eight had mild to moderately active disease affecting only one organ system, and the remaining five patients were in remission. The clinical and laboratory profiles of these patients are shown in Table I. Patients with other autoimmune processes, including rheumatoid arthritis, Sjogren's syndrome, and angioimmunoblastic lymphadenopathy (AILD), had very active disease.

Mononuclear cells were separated from other peripheral blood cells by density centrifugation (17) over Lymphocyte Separation Medium (Bionetics Laboratory Products, Charleston, SC). In some cases, T cells were isolated by rosetting PBMC with sheep erythrocytes, followed by density centrifugation (17). The resulting population contained 94% T cells and <1% B cells when analyzed on a FACS (440; Becton Dickinson Immunocytometry Systems, Mountain View, CA) using fluorescein-labeled OKT 3 (anti–T cell) and anti-Ig reagents as previously described (17). Macrophages were removed from the T cell–depleted pool by plastic adherence to yield a population containing 92% B cells and <2% T cells by FACS analysis.

Preliminary experiments were conducted to determine the optimal conditions for studying protooncogene expression in mitogen-activated human lymphocytes. Cell activation was defined as an increase in RNA and/or DNA synthesis. This was measured by staining lymphocytes with acridine orange and then analyzing their fluorescence on a FACS 440 (Becton Dickinson Immunocytometry Systems) (18). 47% of the T cells and 36% of the B cells were activated under the conditions used in these experiments, consisting of 5 μg/ml Con A for T cells and 25 μg/ml LPS for B cells. All cells were cultured in RPMI supplemented with a mitogenic FCS (10%) at 37°C for 5 h in a humidified 5% CO_2 in air incubator.

Purification of mRNA. Pelleted lymphocytes were ruptured under ribonuclease-inactivating conditions by the method of Chirgwin et al. (19). RNA was separated from other

cellular constituents by centrifugation through a cesium chloride gradient, followed by repeated phenol/chloroform extractions (20). Poly(A)$^+$ RNA was obtained by passing total RNA over an oligo(dT)-cellulose column and eluting with low-salt buffer (21).

Northern Blot Analysis of Protooncogene Expression.   10 µg of poly(A)$^+$ RNA per subject were denatured in 14 mM methylmercury hydroxide, and then electrophoresed on 1.2% agarose gels containing 5 mM methylmercury hydroxide. The RNA was blotted onto *o*-diazophenyl thioether paper, prehybridized, and then hybridized overnight to a nick-translated oncogene probe of sp act 1.4–2.0 × 10^8 cpm/µg in 50% formamide at 42°C (22). After washing at 65°C in 0.1% SSC for 15–60 min, the blots were exposed to Kodak AR-2 film at −70°C in the presence of an intensifying screen.

Several precautions were taken to insure that mRNA samples from different patients could be compared. (a) Ethidium bromide was used to stain each gel before and after transfer, confirming that nearly equal amounts of RNA were blotted onto the paper (see Fig. 4). (b) The analysis of individual patients and controls was repeated several times on different gels with similar results. (c) Preliminary studies using mRNA from the controls was used to establish the duration of blot exposure which produced a linear relationship between mRNA concentration and optical density on x-ray film. (d) These same control mRNAs were included on all blots and used as internal controls for determining the level of protooncogene expression by all other RNA samples. Hybridization intensity was calculated using a Hoefer GS 300 scanning densitometer at a constant setting. The hybridization intensity of all samples was arithmetically normalized such that identical control mRNAs run on different blots gave identical signals when analyzed with the same protooncogene probe. The blots were later stripped twice in 100% formamide at 65°C for 1 h, and analyzed with additional probes.

Purification and Southern Blotting of DNA.   Cell pellets were frozen, lysed with SDS, and then treated with proteinase K (23, 24). The solutions were then extracted with phenol/chloroform, and the supernatants were exposed to RNase. After another treatment with proteinase K and phenol, DNA was recovered by ethanol precipitation and spooling. Eco RI and Bam HI digests (10 µg/lane) were electrophoresed on 0.8% agarose gels. The size and concentration of oncogene-containing bands were assessed by Southern analysis using nick-translated probes.

Cell Cycle Analysis.   The T and B cell populations were analyzed by flow cytometry at various times after mitogenic stimulation for progress through the cell cycle. DNA content of individual cells was measured by staining 2 × 10^6 cells with a hypotonic sodium citrate solution containing 50 µg/ml propidium iodide and 0.1% NP-40 as described previously (25). RNA content was measured on 2 × 10^6 cells, which had been fixed in 70% ethanol and stained with 1% pyronin Y by the method of Shapiro (26). RNA and DNA content were also separately measured by staining cells with acridine orange; under the conditions used, RNA emits green fluorescence and DNA red fluorescence (27). Fluorescence was measured on a FACS 440 using an argon ion laser emitting at 488 nm. The separate emissions of acridine orange were determined by using 30 nm band pass dichroic filters of 530 nm and below vs. 570 nm and above (28). For each sample, data from 50,000 light scatter–gated viable cells were collected.

Cells were deemed to be in G$_1$ when there was a significant increase in RNA content but not DNA content, and in G$_2$, S, or M phase when a significant increase in DNA content was detected (25–28). This was determined by acridine orange staining and separately checked with good agreement in all cases with pyronin Y for RNA and propidium iodide for DNA content. Cell volume was also measured to assess the enlargement of cells after stimulation; this was done with a Coulter counter equipped with a channelizer as described previously (28).

Oncogene Probes.   The c-*myb* probe was a 2.3 kb Hae III fragment of a human *myb* cDNA (J. F. Mushinski, unpublished data). The c-*myc* probe was a 5.5 kb Bam HI fragment from the S107 mouse plasmacytoma line (29). C-*abl* RNA was detected by a 1.2 kb Bgl II fragment derived from cloned Ableson murine leukemia virus (30). The N-*ras* probe, a gift from K. Huppi (National Cancer Institute), was a 2 kb Pvu II fragment from

the SK-N-SH neuroblastoma cell line (31). The c-fos probe was a 3 kb Xho I–Nco I fragment of the pc-fos (human-l) molecular clone (32), and was a gift from S. Bauer (NCI).

Results

Elevated Expression of c-myc in SLE. Expression of the *myc* protooncogene by PBMC was studied in 17 patients with SLE, 7 normal controls, and in 7 patients with autoimmune diseases other than SLE. The top panel of Fig. 1 shows representative results from this analysis with the *myc* probe. Four of the five patients with SLE expressed more c-*myc* RNA than the controls. Elevated c-*myc* expression was also present in the PBMC of the non-SLE autoimmune patients.
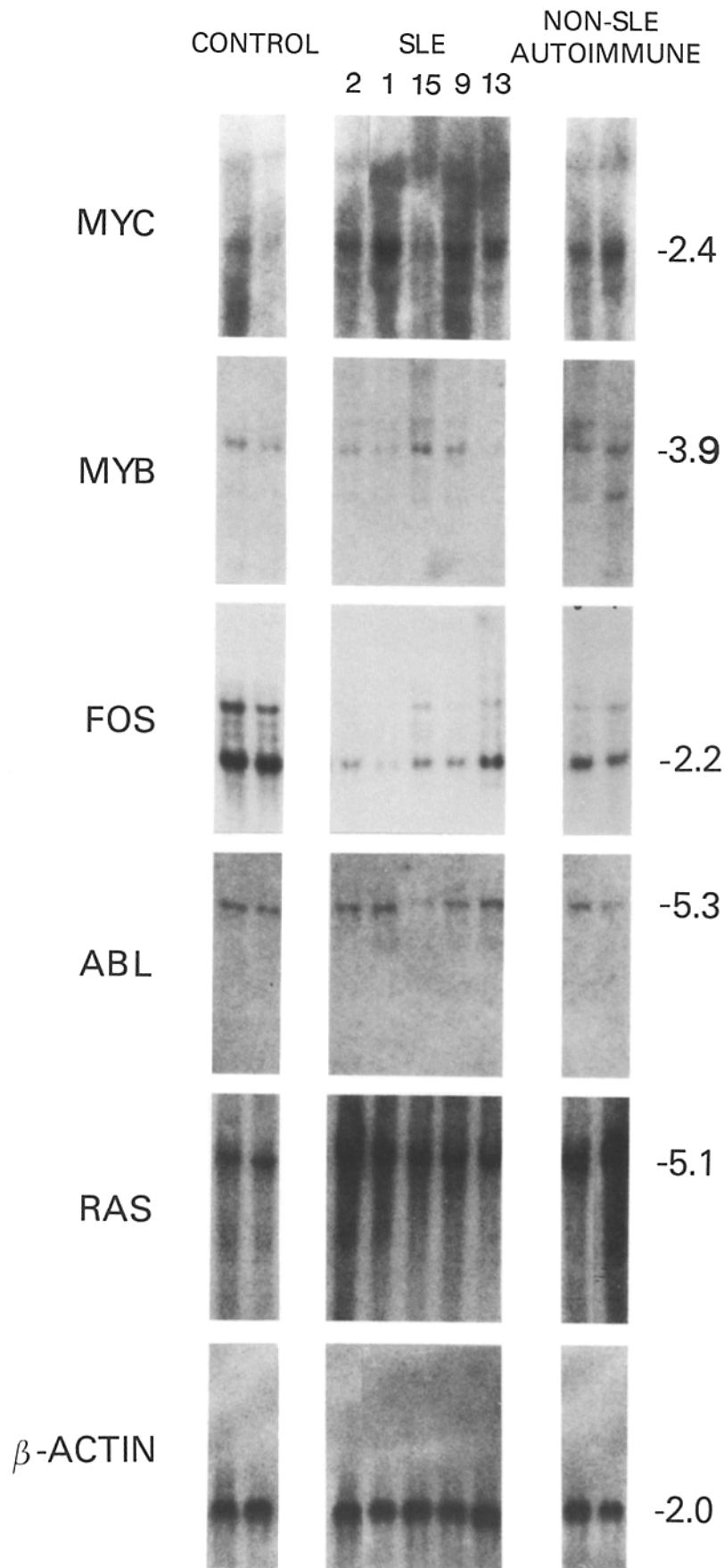
Due to the large number of patients, controls, and standards included in this study, no single Northern gel could contain all of the mRNA samples. To permit comparison between gels, four control mRNAs were included on each. Protooncogene expression was measured by optically scanning autoradiographs of the blots; the results were then normalized to yield equivalent hybridization intensities when identical control mRNAs were run on different blots. Data from all of the blots are summarized in Fig. 2. They show that, on average, PBMC from patients with SLE expressed fourfold more c-*myc* mRNA than did cells from normal volunteers (Fig. 2, first panel). Elevations in c-*myc* mRNA concentration similar in magnitude to those found in SLE were also present in the group of patients with nonlupus autoimmune illnesses (Fig. 2, first panel). This is equivalent to the increase in c-*myc* mRNA concentration found in human tumors such as Burkitt's lymphoma and in mouse plasmacytomas, whose development is associated with abnormal expression of c-*myc* (33, 34).

Decreased Expression of c-myb and c-fos in SLE. Intrigued by the findings with c-*myc*, we examined the expression of two other protooncogenes that encode proteins primarily localized to the nucleus: c-*myb* and c-*fos*. Elevated levels of c-*myb* have been found in tumors of hematopoietic origin (35, 36), and more recently in autoimmune *lpr/lpr* and *gld/gld* mice (5).

PBMC from patients with SLE expressed, on average, threefold less c-*myb* mRNA than did normal controls (second panel, Figs. 1 and 2). This was in contrast to nonlupus autoimmune patients, whose expression of c-*myb* was slightly elevated when compared to the normal volunteers.

C-*fos* encodes a phosphoprotein usually concentrated in the nucleus. Varying c-*fos* expression is associated with different stages of monocyte differentiation in both leukemic and normal individuals (10). When PBMC from lupus patients were compared to controls, the SLE cells contained considerably lower levels of c-*fos* mRNA (third panel, Figs. 1 and 2). Interpretation of these data is complicated by the fact that changes in c-*fos* expression occur within minutes of activation (much faster than the other protooncogenes studied). This is less than the amount of time required to collect and process the mononuclear cell specimens and would allow small differences in sample handling to affect results (see Discussion). The seven patients with nonlupus autoimmune diseases showed a modest decrease in c-*fos* expression when compared to normal controls, which did not reach the level of statistical significance.

Expression of c-abl and N-ras. C-*abl* is the cellular homolog of the transforming element in Abelson leukemia virus (30, 37). The amount of c-*abl* mRNA present

CONTROL   SLE   NON-SLE AUTOIMMUNE

2 1 15 9 13

MYC   -2.4

MYB   -3.9

FOS   -2.2

ABL   -5.3
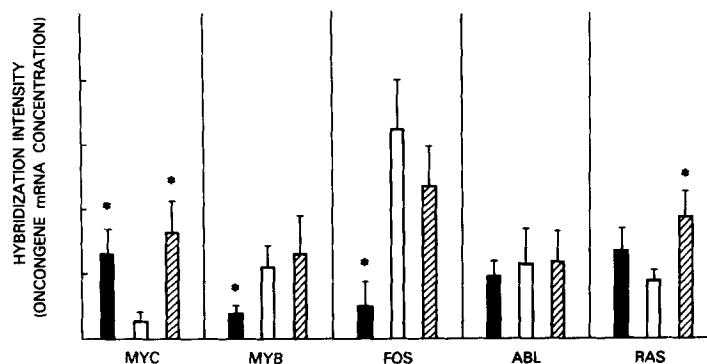
RAS   -5.1

β-ACTIN   -2.0

1296

FIGURE 2. The hybridization intensity (with standard error) of various oncogene probes to poly(A)$^+$ RNA from 17 lupus patients (■), 7 normal controls (□), and 3–7 patients with nonlupus autoimmune diseases (▨), was determined by optically scanning Northern blots. Means of these values (in arbitrary units) are presented graphically. Statistically significant differences between patients and controls ($p < 0.05$) are indicated (*).

in lupus and other autoimmune PBMC was not significantly different from that of normal controls (fourth panel, Figs. 1 and 2). The average concentration of N-*ras* mRNA, which encodes a plasma membrane protein with kinase activity (38), also did not differ significantly between normal controls and patients with SLE (fifth panel, Figs. 1 and 2). However, lupus patients with very active disease expressed significantly elevated concentrations of N-*ras* mRNA when compared to normal volunteers (Table I), as did nonlupus patients with active autoimmune disease (see below).

Protooncogene Expression in Active and Inactive Patients. Of the 17 SLE patients studied, 4 had very active multisystem disease with accompanying laboratory abnormalities, 8 had mild to moderately active disease, and the remaining 5 were in remission. When protooncogene expression in cells from very active patients was compared to those from patients with less active disease, elevations in both c-*myc* and N-*ras* expression were associated with increased disease activity (Table II). These findings suggest that abnormal protooncogene expression may be associated with the development of disease flares. To rule out the possibility that factors other than disease activity might be responsible for the observed differences between more active and less active patients, a chart review was conducted. Specific organ involvement, type of therapy, and drug dose were not found to correlate significantly with protooncogene expression, nor were there other apparent differences between the patient groups.

Cellular Oncogenes in SLE Are Not Rearranged. Chromosomal translocations are frequently associated with the development of malignancies in mice and men (39). This association has been attributed (39) to the capacity of regulatory

FIGURE 1. The hybridization pattern of five oncogene probes to mRNA samples from two controls, five lupus patients, and two patients with nonlupus autoimmune diseases (rheumatoid arthritis and angioimmunoblastic lymphadenopathy) is shown. For each oncogene probe, a single gel containing all poly(A)$^+$ RNA samples was run, blotted, and hybridized so that conditions would be standard. Numbers above the lanes from the lupus patients correspond to the patient profiles presented in Table I. The transcript sizes of the protooncogene mRNAs are shown in kb along the right-hand border. The expression of actin mRNA, whose expression does not vary significantly during the cell cycle, is shown as a control.

TABLE II

Effect of Disease Activity on Oncogene Expression

Group	Oncogene									
	myc		myb		fos		abl		ras	
	Mean	p	Mean	p	Mean	p	Mean	p	Mean	p
Very active SLE	224	0.03	43	0.02	142	0.001	74	>0.10	272	0.02
Less active SLE	77	0.05	36	0.01	74	<0.001	98	>0.10	89	>0.10
Normal controls	29		110		646		113		85	

Mean protooncogene expression by patients with very active disease (n = 4) and less active disease (n = 13) was compared to normal controls (n = 7). Statistically significant differences, indicated by p values <0.05, were determined using the student's t test.
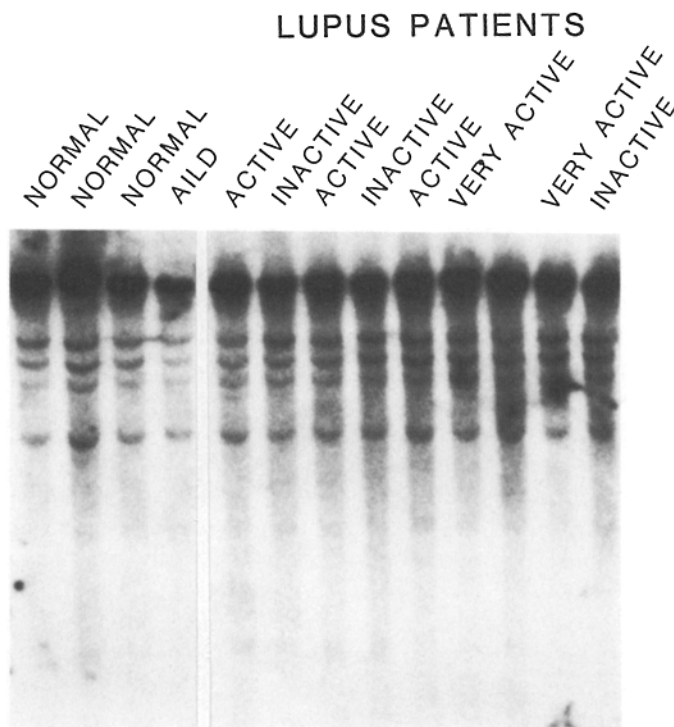

FIGURE 3. Southern blot of Bam HI digests of DNA from lupus patients and normal controls hybridized with N-*ras* probe. Gene rearrangement was not found using this or other oncogene probes.

elements at the site of translocation to abnormally influence protooncogene expression. Southern blots were used to determine whether altered levels of cellular oncogene expression in patients with SLE were also associated with gene rearrangement or with gene amplification. DNA was isolated from PBMC of patients and normal controls and then digested with the Eco RI or Bam HI restriction endonucleases. Fragments hybridizing with probes for c-*myc*, c-*myb*, c-*fos*, c-*abl*, and N-*ras* were compared for size and intensity to normal controls on the same blot. In no case was there evidence of specific protooncogene rearrangement among the SLE patients, nor was there a difference in gene dosage. Fig. 3

shows results with N-*ras*; the other oncogene probes similarly revealed no evidence of gene amplification or rearrangement.

Protooncogene Expression in Mitogen-activated Lymphocytes.   To better interpret the observed abnormalities of protooncogene expression in patients with autoimmune diseases, we studied whether cellular activation influenced c-*onc* gene mRNA levels in normal human lymphocytes. PBMC were separated into relatively pure populations of T and B cells, which were then stimulated with mitogen and cultured for various times in vitro. mRNA isolated from these cells after 0, 2, 5, and 18 h in culture was analyzed for the expression of those cellular oncogenes whose mRNA levels were significantly different in the PBMC of patients vs. normal controls.

Changes in the concentration of c-*myc* mRNA have been detected after mitogenic stimulation of mouse spleen cells (9), hematopoietic cells (10), and fibroblasts (40, 41). In addition, high levels of this oncogene's transcript are found in a variety of neoplasms (reviewed in 34). Thus it was not surprising that the stimulation of both T and B lymphocytes induced an elevation in the level of c-*myc* mRNA (Fig. 4). From a very low baseline in unstimulated cells, the concentration of c-*myc* RNA rose detectably by 1.5 h after mitogen treatment, peaked at 5 h and declined slightly by 18 h. These findings are in keeping with previous reports (9, 40, 41) that c-*myc* expression peaks ~2 h after stimulation and decreases rapidly thereafter.

c-*myb* expression was also analyzed. High levels of this cellular oncogene's transcript are associated with hematopoietic tumors including T cell lymphomas and B cell leukemias (35, 36), and with certain autoimmune states (5). Our study revealed that increased c-*myb* expression was induced by the Con A activation of purified human T cells. The concentration of this protooncogene's transcript remained at background levels through 1.5 h in culture then rose to a peak at 5 h and persisted through 18 h (Fig. 4). In contrast, mitogen-activated B cells showed no such rise in c-*myb* RNA. This marks an important difference in the pattern of c-*onc* gene expression between activated human T and B lymphocytes.

It could be argued that LPS is not as strong a mitogen for human B cells as Con A is for T cells. The observed induction of other oncogenes in LPS treated B lymphocytes makes such an explanation unlikely. In addition, significant B cell proliferation under these conditions was documented both by conventional [^{3}H]-thymidine incorporation studies (data not shown) and by analysis of RNA and DNA content using cell cycle analysis (see Table III).

Expression of the c-*fos* gene has been linked to differentiation of monocytes from leukemic and normal individuals (10), and has been shown to be a very early event after activation of resting 3T3 fibroblasts (11, 40). In our studies, normal unstimulated PBL showed a high resting level of c-*fos* mRNA (Figs. 1 and 2). Mitogen activation of either T or B cells induced a >10-fold decline in the concentration of c-*fos* mRNA within 1.5 h and its reduction to undetectable levels at later time points (Fig. 4).

It is possible but unlikely that changes in c-*fos* expression were induced by the procedures used to purify the lymphocytes. These techniques alone did not lead to cellular proliferation or the expression of other protooncogenes (Fig. 1). In addition, high levels of c-*fos* RNA were found in aliquots of cells isolated at every
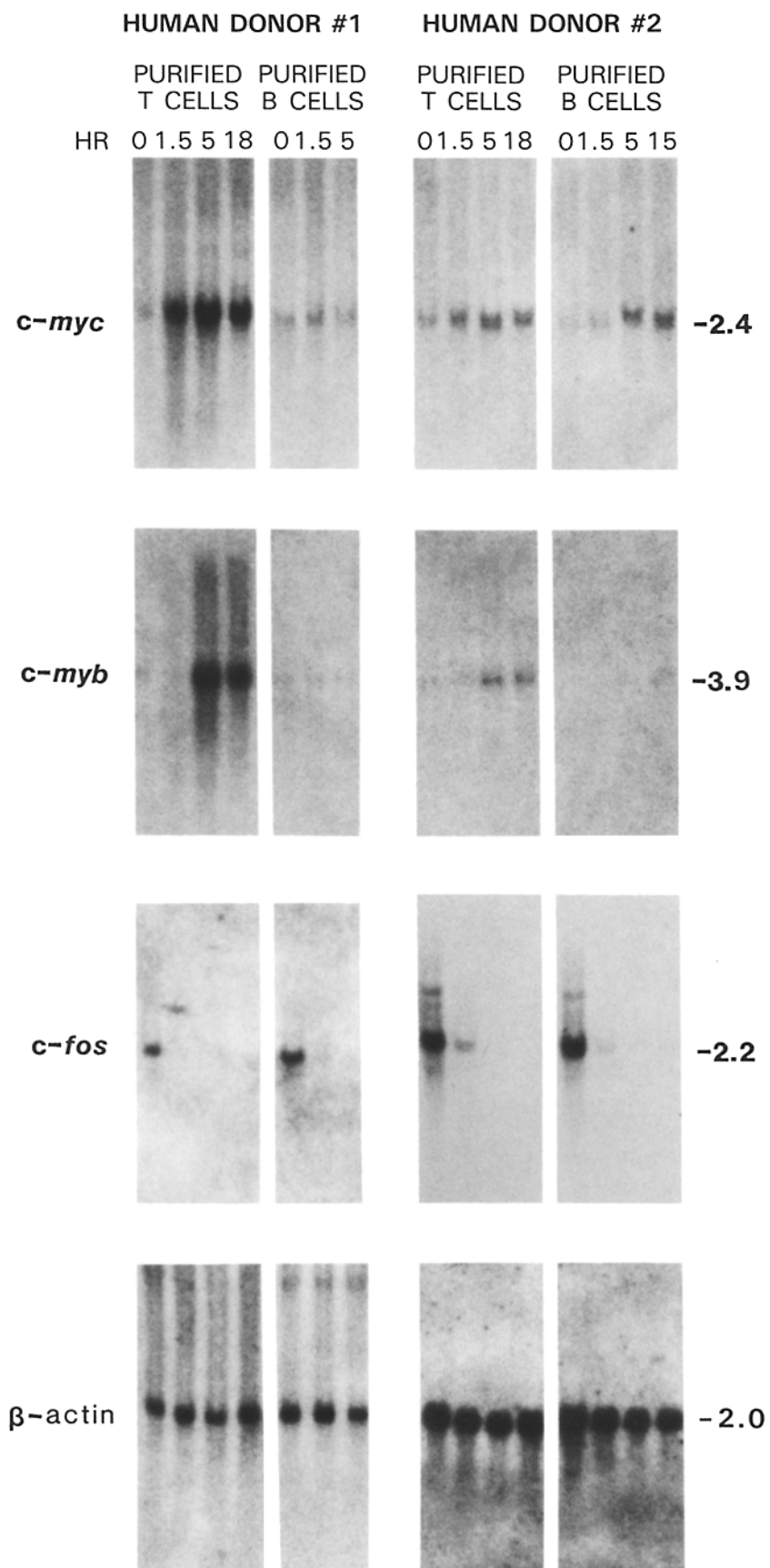
HUMAN DONOR #1

HUMAN DONOR #2

PURIFIED T CELLS   PURIFIED B CELLS

PURIFIED T CELLS   PURIFIED B CELLS

HR   0 1.5 5 18   0 1.5 5   0 1.5 5 18   0 1.5 5 15

c-*myc*   −2.4

c-*myb*   −3.9

c-*fos*   −2.2

β−actin   −2.0

1300

TABLE III

Cell Cycle Analysis of Activated B and T Lymphocytes

Lymphocytes	Hours post-stimulation	Percent of cells in each stage of cell cycle*		
		G_0	G_1	S, G_2, M
B	0	96.4	2.3	1.3
B	1.5	97.9	1.5	1.6
B	5	87.5	11.2	1.3
B	24	82.8	12.0	5.2
B	48	63.9	11.1	25.0
T	0	94.6	4.3	1.1
T	1.5	97.2	1.3	1.5
T	5	94.8	3.2	2.0
T	24	86.5	11.2	2.3
T	48	42.4	16.0	41.6

* Cells were stained with acridine orange and their content of RNA and DNA was determined by two-color flow cytometry. In comparison to resting G_0 cells, lymphocytes that showed an increase in RNA but not DNA content were defined as being in G_1 (11). Cells in S, G_2, and M were those expressing increased levels of DNA. These designations were confirmed by independent analysis of RNA and DNA content using pyronin Y and propidium iodide, respectively (8, 9).

step of the purification procedures, and in an unprocessed human spleen (data not shown). Assuming that the short half-life of the c-fos transcript (11, 40) was not altered by the collection procedure, these findings argue against the possibility that expression of this protooncogene was induced by the purification protocol.

Cell Cycle Analysis of Activated Lymphocytes. Mitogen-activated T and B lymphocytes were stained at various times with nucleic acid–binding dyes and then analyzed for RNA and DNA content using a FACS. After 48 h of in vitro culture, 36.1% of B cells and 57.6% of T cells had been stimulated to leave G_0 and enter a later stage of the cell cycle (Table III). However, after 5 h in culture (when maximal changes in protooncogene expression were found), only 12.5% of B cells and 5.2% of T cells had cycled from G_0 to G_1, as defined by a significant increase in RNA content (Table III and analysis of cell volume). Furthermore, analysis of DNA content showed that very few cells had progressed to S phase within the first 24 h of lymphocyte activation (Table III). In toto, these data show that, while mitogenic stimulation induces a large proportion of T and B cells to progress through the cell cycle (consistent with results from other investigators [26, 27]), the characteristic changes in total cellular RNA and DNA content that accompany transition into G_1, S, G_2, and M lag behind alterations in the expression of specific cellular oncogenes.

FIGURE 4. Protooncogene expression by activated normal T and B lymphocytes. Lymphocytes were purified from apheresis samples and then stimulated with mitogen. Northern blots of mRNA isolated from these cells at 0, 1.5, 5, and 18 h poststimulation were hybridized to nick-translated cellular oncogene probes. The sizes of major transcripts for each protooncogene are shown on the left of each panel. Expression of actin mRNA is shown as a control.

Discussion

The concentration of protooncogene mRNA present in PBMC of patients with autoimmune diseases was compared to that of normal controls. The cells from patients with SLE had significantly more c-myc RNA than normal, and those from patients with active SLE expressed significantly elevated levels of N-ras RNA. Diminished levels of c-myb and c-fos RNA were also found in the SLE group. These findings were compared to cellular oncogene levels in patients with nonlupus autoimmune diseases, and to mitogenically activated lymphocytes from normal donors.

While the level of c-myc RNA was highest in patients with active disease, it was also significantly elevated in clinically stable patients. We also found that PBMC from patients with Sjogren's syndrome, rheumatoid arthritis, and angioimmunoblastic lymphadenopathy expressed elevated c-myc RNA. Purposeful stimulation of normal PBMC resulted in an increase in c-myc mRNA concentration in both T and B cell populations. These findings suggest that lymphocyte activation, induced by mitogen in vitro or by autoimmune disease in vivo, leads to an increase in c-myc expression. These data are consistent with the increased proliferation of B cells reported (42) in patients with stable SLE, even though they may not have increased numbers of immunoglobulin-secreting cells (43, 44).

N-ras mRNA was not found to be significantly elevated in the SLE group as a whole, but was increased in patients with active disease. A similar result has been reported in mice with lupus-like disorders. The B cells of NZB and BXSB mice had elevated N-ras and c-myc expression. When congenic NZB.xid and BXSB.xid mice were bred and tested (the xid gene markedly retarding B cell maturation and the production of autoantibodies [45]) expression of these protooncogenes was subnormal (5).

In contrast to the elevation in c-myc and N-ras, c-myb and c-fos mRNA levels were significantly decreased in patients with both active and inactive SLE. Since c-fos expression falls rapidly following lymphocyte activation (Fig. 5), these data suggest that some degree of PBMC stimulation is present in these patients.

An explanation for low c-myb expression presents itself in the data on mitogen stimulated lymphocytes. High c-myb RNA appeared to be a feature of stimulated T cells, but not B cells (Fig. 4, second panel). Therefore, if an individual's disease were characterized by the specific activation of B cells, one might anticipate low c-myb and high c-myc RNA levels. This pattern is found in patients with SLE but not in those with other autoimmune diseases (where decreased expression of c-myb was not found). Whether this observation will be of use in diagnosing patients with clinically undifferentiated rheumatic disease will require further study.

Our results also show that mitogenic activation of human lymphocytes induces an orderly sequence of change in the level of expression of specific cellular oncogenes (Fig. 5). Shortly after activation, the concentration of c-fos message declines. At ~1.5 h, an increase in the expression of c-myc occurs, reaching a maximum at 5 h and persisting for at least 18 h. Also at 5 h, an increase in c-myb expression is detected in the T cell pool but not among B cells. These changes precede the rise in total cellular RNA content associated with transition from a resting G_0 to an activated G_1 state.

Cell cycle analysis of the T and B cell populations showed that a large
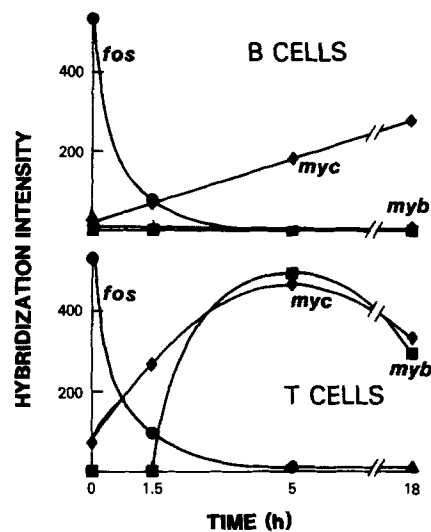
FIGURE 5. Protooncogene expression in activated normal T and B lymphocytes over time. Autoradiographs of the northern blots presented in Fig. 4, whose hybridization intensities were within the linear response range of the Hoefer GS 300, were selected for further analysis. The radiographs were optically scanned at the level of each protooncogene, and hybridization intensity was used to determine protooncogene expression, which was plotted as a function of time.

proportion of cells in both populations responded to mitogenic stimulation within 48 h. At 1.5 h, however, there was no significant increase in RNA content among the activated lymphocyte populations, and only 5% of T cells and 12% of B cells had entered G_1 by 5 h (26, 27). None reached S phase by this time. These data suggest that protooncogene expression changes before a cell's transition through the cell cycle. This is especially clear in the case of c-fos, whose expression decreased fivefold within 1.5 h of stimulation. While it might be argued that the elevated expression of other protooncogenes was moderated by the small number of cells leaving G_1 by 5 h, this seems unlikely, since the number of cells leaving G_0 remained high for over 24 h, yet the levels of myb and abl RNA fell to near resting levels by 18 h.

The sequential activation of c-fos, c-myc, and then c-myb (Fig. 5) supports the contention that such cellular oncogenes perform vital cellular functions and are specifically involved in growth and differentiation (9). Further, it is consistent with the concept of a cascade whereby altered expression of one protooncogene is required before changes in the expression of another protooncogene may occur (9, 40). All cellular oncogenes are not involved in this cascade, however. The stimulation of T and B lymphocytes led to no change in the level of N-ras mRNA in our study (data not shown). Our finding that c-myb expression increased in activated T cells but not in activated B cells demonstrates that, even among closely related tissue types, cellular activation involves precise and independent regulation of the expression of specific cellular oncogenes. This understanding of protooncogene expression by normal lymphocytes has cleared the way for the interpretation of abnormal cellular oncogene expression in autoimmune disease states.

These studies are an attempt to determine whether specific genes are differentially expressed in patients with autoimmune disease (5). Our results indicate that abnormalities in protooncogene mRNA levels are frequently found in patients with SLE. Whether these abnormalities stem from intrinsic defects in gene regulation, increased lymphocyte activation, or expansion of particular lymphocyte subpopulations in diseased individuals has not yet been determined. These alternatives are not mutually exclusive. We believe that future studies of differential gene expression will better define the molecular bases of autoimmune diseases. Such studies may include the isolation of genes associated with autoimmunity, followed by the characterization of their protein products and analysis of the mechanism(s) by which such products lead to abnormal cellular activation and differentiation.

Summary

PBMC from patients with autoimmune diseases and from normal controls were studied for the expression of several cellular oncogenes. Gene expression was assessed by Northern blot analysis of poly(A)$^+$ RNA obtained from leukapheresis samples. Patients with SLE expressed significantly more c-myc protooncogene RNA than did normal controls. Increased expression of the N-ras protooncogene was found in that subset of patients whose autoimmune disease was very active. Cells from individuals with SLE, but not from those with other autoimmune illnesses, showed significantly decreased levels of the c-myb and c-fos protooncogenes.

To examine the implications of these findings, B and T cells were purified from apheresis samples donated by normal volunteers. When mitogen was used to activate the B cells in vitro, their pattern of protooncogene expression changed to resemble that found in freshly isolated cells from lupus patients. These results suggest that the differences detected in the expression of protooncogenes by patients with SLE may be due to the abnormal activation of their B cells in vivo. The pattern of protooncogene expression found in patients with other autoimmune illnesses is consistent with the activation of additional cell types in those diseases.

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