Signal Transducer and Activator of Transcription-3 (STAT3) Is Constitutively Activated in Normal, Self-renewing B-1 Cells but Only Inducibly Expressed in Conventional B Lymphocytes

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Summary

Cytokine and growth factor receptor engagement leads to the rapid phosphorylation and activation of latent, cytosolic signal transducers and activators of transcription (STAT) proteins, which then translocate to the nucleus where they regulate transcriptional events from specific promoter sequences. STAT3 expression in particular has been associated with Abl, Src, and HTLV-1 transformation of normal cells. B-1 lymphocytes are self-renewing, CD5+ B cells that display a propensity for malignant transformation and are the normal counterpart to human chronic lymphocytic leukemias. Further, B-1 cells are characterized by aberrant intracellular signaling, including hyperresponsiveness to phorbol ester PKC agonists. Here we demonstrate that B-1 lymphocytes constitutively express nuclear activated STAT3, which is not expressed by unmanipulated conventional (B-2) lymphocytes. In contrast, STAT3 activation is induced in B-2 cells after antigen receptor engagement in a delayed fashion (after 3 h). Induction of STAT3 is inhibited by both the serine/threonine protein kinase inhibitor H-7 and the immunosuppressive drug rapamycin and requires de novo protein synthesis, demonstrating novel coupling between sIg and STAT proteins that differs from the classical paradigm for STAT induction by cytokine receptors. The inability of prolonged stimulation of conventional B-2 cells with anti-Ig, a treatment sufficient to induce CD5 expression, to result in sustained STAT3 activation suggests that STAT3 is a specific nuclear marker for B-1 cells. Thus, STAT3 may play a role in B cell antigen-specific signaling responses, and its constitutive activation is associated with a normal cell population exhibiting intrinsic proliferative behavior.

The 67-kD pan-T cell surface glycoprotein, CD5, was first detected on the surface of human and murine B cell tumors and subsequently found to specify a subset of normal B lymphocytes in both species (1). CD5+ (or B-1) B lymphocytes are mature B cells that predominate early in life, decline in relative number as the animal matures, and, in mice, become confined to the peritoneal cavity, with few, if any, present in the peripheral lymph nodes (2). Functionally, B-1 cells contribute a disproportionately large fraction of serum Ig, specifically of the μ, α, and γ classes. These Igs are noted to express germline encoded specificities, with little somatic mutation and N-insertion and may be involved in the regulation of idiotype expression (3, 4).

B-1 cells have been linked to both autoantibody production and the pathogenesis of autoimmune disease as well as malignancy (2, 5). CD5+ B cells have been found to be enriched sources of autoantibody-producing cells specific for various self-antigens, and several mouse strains that develop autoimmune pathology have elevated numbers of splenic and peritoneal CD5 B cells (6, 7). Adoptive transfer experiments have demonstrated that B-1 cells have self-renewing capacity (8), and in vitro, these cells are readily immortalized in culture without the use of exogenously induced transformation (9). Coupled with their hyperresponsiveness to PMA stimulation and their inability to enter S phase after sIg cross-linking (10, 11), these observations suggest that B-1 cells differ from B-2 cells in their biochemical makeup in ways that may contribute to autoantibody secretion and unregulated growth.

Signal transducers and activators of transcription (STAT) proteins were first characterized by studying signaling in re-
response to interferon and have since been implicated in cellular responses to a plethora of cytokines and growth factors (12, 13). STAT signaling involves the activation of the JAK/tyk family of tyrosine kinases that are believed to be associated with unliganded cytokine receptors and to phosphorylate latent cytoplasmic STAT proteins upon ligand binding (14). Phosphorylated STATs dimerize via interactions between their SH2 domains (15), allowing nuclear translocation and DNA binding activity specific for distinct sequence elements in cytokine and growth factor–stimulated genes. We have previously shown that mitogenic stimulation through surface Ig in B-2 cells induces the activation of STAT proteins (16). This observation, coupled with the association of STAT3 with abnormal cell growth and transformation (17–20), led us to compare the status and activational responses of STAT3 proteins in B-1 and B-2 cells. Our results indicate that the nuclear expression of activated STAT proteins differs between B-1 and B-2 cells and that the STAT protein profile may be a distinguishing molecular feature of the B-1 cell phenotype.

Materials and Methods

Animals. Male BALB/cByJ mice at 8–14 wk of age were obtained from The Jackson Laboratory (Bar Harbor, M E). M ice were housed at least 1 wk before experimentation. Mice were cared for and handled at all times in accordance with National Institutes of Health and institutional guidelines.

B Cell Purification. B-1 lymphocytes were prepared by negative selection from peritoneal wash-out cells as previously described (21). B-2 cells were purified from spleen cells of B-12-wk-old naive mice by depletion of T cells using treatment with anti-Thy 1.2 antibody plus rabbit complement and depletion of macrophages by overnight culture on plastic petri dishes, as previously described (22). RBC and nonviable cells were removed by sedimentation over Lympholyte M (Cedarlane, Ontario, Canada). The resulting B cells were cultured at 37°C with 5% CO₂ in RPMI 1640 medium (BioWhittaker, Walkersville, MD) supplemented with 5% heat inactivated fetal bovine serum (Sigma Chem. Co., St. Louis, MO), 10 mM Hepes (pH 7.2), 50 µM 2-ME, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. In some experiments, B-1 cells were prepared in the presence of serum-free RPMI 1640 medium containing 1% bovine serum albumin and cultured in serum-free AIM-V medium (GIBCO BR L, Gaithersburg, MD).

Nuclear extracts and electrophoretic mobility shift assay. Nuclear extracts from untreated or stimulated primary B cells were prepared using 430 mM NaCl at pH 7.9 as previously described (22), except that 1 mM sodium orthovanadate was added to all extraction buffers. Protein concentrations were determined by the Bradford method (23) (Bio-Rad, Hercules, CA). Nuclear extract protein was incubated with 32P-labeled double-stranded oligonucleotide containing the high-affinity SIE (m67) derived from the c-fos gene (24) (5' GTGCAATTTCCCGTAAATCTCTTGTCAACT3') for 20 min before electrophoresis on nondenaturing 5% polyacrylamide gels. Binding reactions contained 1 µg poly(dI-dC) and 2.0 µg salmon sperm DNA. For competition analysis, 20-fold excess unlabeled SIE or NF-AT oligonucleotide (25) was added to binding reactions before addition of nuclear protein and electrophoresis. Supershift immunoinhibition analysis was performed by addition of 1 µl anti-p91N or antiphosphotyrosine STAT1 for an additional 30 min at 4°C after the 20-min electrophoretic mobility shift assay (EMS A) binding reaction.

Western blotting. Nuclear extracts (5 µg) was resolved by 7.5% SDS-PAGE, transferred to nitrocellulose and blocked with 5% nonfat powdered milk in wash buffer (20 mM Tris, pH 7.6, 0.14 M NaCl, and 0.1% Tween-20; TBS-T) overnight at 4°C. The nitrocellulose filters were then probed with antiphosphotyrosine STAT1 (26), or with antiphosphotyrosine STAT3 antisera, generated by immunization of rabbits with a synthetic peptide containing amino acids 696–709 of human STAT3, with phosphotyrosine at position 705, which was conjugated to bovine serum albumin. Blots were incubated for 1 h at room temperature with antibody in 3% BSA–TBS-T. After washing, blots were developed by ECL.

Shift-Western. EMSA was performed as described above except 5 µg of nuclear extracts were added to 1.5 × the normal amount of labeled oligonucleotide and electrophoresed on 5% polyacrylamide gels. SIE-binding proteins were separated from retarded labeled oligonucleotide by electrophoretic transfer from the native gel to nitrocellulose paper. Labeled oligonucleotide was detected bound to DE-81 paper (Whatman, Hillisboro, OR) placed under the nitrocellulose filter and on top of Whatman filter paper. After transfer, the nitrocellulose filters were blocked and Western blotted as above and the DE-81/W hatman filters were dried for 10 min before autoradiography.

Reagents. F(ab')₂ fragments of goat anti–mouse IgM (Jackson Immunoresearch, Inc., West Grove, PA) were used at a concentration of 15 µg/ml. PMA (Sigma) was used at 100 ng/ml. Cells were stimulated with murine recombinant IFN-γ (5 ng/ml) or IL-6 (10,000 U/ml), both from Genzyme (Cambridge, MA), rabbit anti-STAT3 antibody to the NH₂-terminal domain of p91 anti-p91N was the kind gift of Dr. C. Schindler (Columbia University, New York). Rabbit antisera specific for only the tyrosine-phosphorylated form of STAT1 was generated by immunization of rabbits with a peptide designed from the STAT1p91 protein containing phosphorylated Tyr 701 (26). Rapamycin was used at 20 ng/ml, dissolved in ethanol and 1-(5-isouquinolinesulfonyl)-2-methylpiperazine (H-7) and N-(2′-guanidinoethyl)-5-isouquinolinesulfonamide (HA-1004) (LC Laboratories, Woburn, MA) were used at 25 µM.

Results and Discussion

To determine whether the unusual growth characteristics of B-1 cells are accompanied by differences in the regulation of STAT proteins, we compared STAT DNA-binding activities between resting B-1 and B-2 cells. Nuclear extracts from untreated B-1 cells formed protein–DNA complexes with the high-affinity sis-inducible element (SIE) of the c-fos gene (27), a recognized STAT-binding site (1), as detected by EMSA (Fig. 1A). The major B-1 cell-specific SIE-binding activity was observed to co-migrate with the IL-6-stimulated sis-inducible factor (SIF) A binding complex, with a smaller amount co-migrating similarly to the IFN-γ-induced SIF C complex (24, 26–30). In contrast, no SIF A, and little SIF C activity was detected in nuclear extracts obtained from unmanipulated B-2 cells.

The B-1 cell complexes were competed by unlabeled SIE-containing oligonucleotide but not by the consensus binding site for the nuclear factor of activated T cells (NF-AT) (Fig. 1B), indicating that these complexes are specific for the SIE. The constitutive expression of SIE complexes in

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B-1 cells was not the result of serum induction in vitro, as these complexes were still formed from nuclear extracts prepared from B-1 cells purified and cultured in serum-free medium and were not inducible upon subsequent serum treatment (Fig. 1C). Antibody to the NH₂-terminal region of STAT1α (anti-p91N), which has been shown to recognize STAT3 induced by ciliary neurotrophic factor (CNTF) (31), disrupted SIF A induced by IL-6 in B cells (Fig. 2A). The constitutively expressed B-1 SIF A-like complex was also disrupted by anti-p91N but not by an antibody to phosphotyrosine (anti-p91N), which has been reported to induce the tyrosine phosphorylation of two STAT3 isoforms, p88 and p89 (31). These isoforms, whose phosphorylation is also inducible by IL-6 (29, 32, 33), have been termed STAT3β (p88), for faster migrating STAT3, as determined by immunoblotting material obtained from the native gel (Fig. 2B, bottom). As a control, phosphotyrosine (anti-p91N) formed supershifted complexes with SIF A in both IL-6-stimulated B cells and untreated B-1 cells which were apparent in longer exposures and showed identical electrophoretic mobilities; antiserum to STAT4, 5, or 6 failed to react with the B-1 cell SIF A complex in EMSA supershift assays or with B-1 cell nuclear extracts Western blotting experiments, and antibody that supershifted STAT1 activated by IFN-γ immunoinhibited supershifted only a small amount of the SIF C complex of B-1 cells (data not shown). Because STAT3 is only known to form homodimers or to heterodimerize with STAT1, these results strongly suggest that B-1 cells differ from B-2 cells in the basal nuclear expression of STAT3 homodimers that comprise the SIF A nucleoprotein complex.

To assess the contribution of phosphorylated STAT3 to the SIF A complex constitutively present in B-1 cells, we performed Shift-Western experiments, using nuclear extracted protein, the SIE-containing oligonucleotide, and anti-phosphotyrosine (anti-p91N) antibody to supershift STAT1 activated by IFN-γ or CNTF (Fig. 2C). Anti-p91N formed supershifted complexes with SIF A in both IL-6-stimulated B cells and untreated B-1 cells (Fig. 2C, top). Nuclei were incubated with the SIE oligonucleotide before Western blotting with antiserum specific for STAT3 phosphorylated on tyrosine (no DNA control) was observed to migrate more slowly in the gel, distinguishing it from SIE-bound STAT3 (data not shown). These results confirm that STAT3 is constitutively present in B-1 nuclei, as indicated by the supershifting experiments outlined above and show that it is present as a tyrosine phosphorylated protein in the SIF A complex.

We further examined the phosphorylation status of STAT3 in nuclear extracts from B-1 and B-2 cells. CNTF has been reported to induce the tyrosine phosphorylation of two STAT3 isoforms, p88 and p89 (31). These isoforms, whose phosphorylation is also inducible by IL-6 (29, 32, 33), have been termed STAT3β (p88), for faster migrating and STAT3α (p89), for slower migrating (32, 34). Immunoblotting with antiserum specific for STAT3 phosphorylated on tyrosine (anti-p91N) showed that B-2 cells express little nuclear phosphotyrosine (anti-p91N) antibody to supershift STAT1 activated by IFN-γ or CNTF (Fig. 2C, top). Nuclei were incubated with the SIE oligonucleotide before Western blotting with antiserum specific for STAT3 phosphorylated on tyrosine (no DNA control) was observed to migrate more slowly in the gel, distinguishing it from SIE-bound STAT3 (data not shown). These results confirm that STAT3 is constitutively present in B-1 nuclei, as indicated by the supershifting experiments outlined above and show that it is present as a tyrosine phosphorylated protein in the SIF A complex.

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nuclear extracts were found to contain roughly equal levels of phosphotyrosine\(^{705}\)STAT3 and STAT3, respectively, which co-migrated with IL-6-induced phosphotyrosine\(^{705}\)STAT3 from B-2 cells (Fig. 2 C). In addition, immunoblotting untreated B-1 cell nuclear extracts with antibody specific for STAT1 phosphorylated on tyrosine\(^{701}\) detected activated STAT1 of the p91 isoform, which was not present in nuclear extracts from untreated B-2 cells but was inducible by IFN-\(\gamma\) treatment (Fig. 2 D). Thus, the B-1-specific expression of SIF A correlates with the presence of the phosphotyrosine\(^{705}\) forms of STAT3, and STAT3 in nuclei from unstimulated B-1 cells, and B-1 cells also constitutively express a small amount of activated p91-STAT1.

The presence of SIF A and phosphotyrosine\(^{705}\)STAT3 in B-1 cells could not be due to macrophage contamination, because purified macrophages isolated by adherence during B cell purification from the same animals did not contain nuclear SIF A or phosphotyrosine\(^{705}\)STAT3, and histologic examination of B-1 populations revealed less than 2% macrophage contamination (data not shown). Further, overnight incubation of B-1 cells with neutralizing antibody to IL-10 before preparation of nuclear extracts did not result in diminution of the SIF A complex observed by EMSA (data not shown), suggesting that an IL-10 autocrine loop

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**Figure 2.** Immunoreactivity of B-1 cell SIF A with STAT3-specific antisera, and detection of constitutively expressed B-1 cell STAT1 and 3 isoforms. (A) EMSA supershift/immunoinhibition analysis was performed using nuclear extracts from untreated B-1 cells (M) or B-2 cells stimulated with IL-6 and antibody to the NH\(_2\)-terminal region of STAT1, which has been shown to supershift STAT3 activated by IL-6 or CNTF (\(\alpha\)-p91N). Antiserum specific for phosphorylated STAT1 (\(\alpha\)-ptyrSTAT1) (26) was used as a control. Arrows indicate the positions of SIF A, B, and C. (B) B-1 cells were incubated in medium alone (−) and B-2 cells were either incubated in medium alone or were stimulated with IL-6 (1,000 U/ml) for 15 min, after which nuclear extracts were prepared. Nuclear extracted protein was analyzed by EMSA using the \(^{32}\)P-labeled SIE-containing oligonucleotide probe and 5% nondenaturing PAGE. Retarded radiolabeled oligonucleotide was separated from DNA-binding proteins by a Shift-Western procedure, using electrotransfer to Whatman DE-81 paper and nitrocellulose filter paper, respectively. The SIE-containing oligonucleotide was visualized by autoradiography (top) and DNA-binding protein was immunoblotted with STAT3 phosphotyrosine\(^{705}\)-specific antibody and detected by ECL (bottom). The positions of the specific SIE-binding nucleoprotein complexes and immunoblotted phosphotyrosine\(^{705}\)STAT3 are indicated (arrows). (C) Immunoblot analysis of nuclear extracts from unstimulated B-2 or B-1 cells (left) or B-2 cells that were incubated with medium alone (M) or stimulated with IL-6 (B-2/IL-6; 1,000 U/ml for 15 min) (right), probed with antibody specific for STAT3 phosphorylated on tyrosine 705. Arrows indicate fast (p88) and slow (p91) migrating forms of STAT3. To test for equal loading of lanes, the blot was reprobed with an antibody to the constitutively expressed nuclear transcription factor, CREB (UBI, Lake Placid, NY). (D) Immunoblot analysis of nuclear extracts from untreated (M) or B-2 cells (lanes 1 and 2) or B-2 cells stimulated with IFN-\(\gamma\) (5 ng/ml for 15 min; lane 3), probed with antibody specific for phosphotyrosine\(^{705}\)STAT3 (26). Arrows indicate 91- and 84-kD isoforms of STAT1.

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**Figure 3.** Inducible nuclear expression of STAT3 in B-2 cells treated with anti-Ig. (A) Delayed nuclear expression of SIF A in anti-Ig-stimulated B-2 cells. EMSA analysis for SIE-binding activity was carried out using nuclear extracts prepared from B-2 cells incubated in medium alone (−) or stimulated with F(ab\(^9\))\(_2\) fragments of goat anti-mouse IgM (alg; 15 \(\mu\)g/ml), as indicated. Arrows indicate positions of nucleoprotein complexes containing SIF A, B, and C. (B) Immunoreactivity of anti-Ig-induced B-2 cell SIF A with a STAT3-specific antiserum. Gel mobility supershift/immunoinhibition analysis was performed using nuclear extracts from B-2 cells stimulated with F(ab\(^9\))\(_2\) GaM IgM (alg) for 3 h and added to radiolabeled SIE-containing oligonucleotide before addition of antiserum specific for STAT3 (\(\alpha\)-p91N) or for phosphorylated STAT1 (\(\alpha\)-ptyrSTAT1); see legend to Fig. 1. Arrows indicate the position of nucleoprotein complexes containing SIF A, B, and C.
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Figure 4. Unique features of the surface Ig-mediated STAT3 signaling pathway. (A and B) Induction of tyrosine phosphorylated STAT3 by anti-Ig in B-2 cells requires serine/threonine phosphorylation. Nuclear extracts were obtained from B-2 cells stimulated for 3 h with F(ab′)2 GaM IgM (aIg) alone or with aIg in the presence or absence of either the serine/threonine kinase inhibitor H-7, or its less active structural analogue, HA1004 (both at 25 μM; LC Laboratories) and probed with antibody specific for phosphotyrosine705STAT3 (p89). Arrows indicate fast (p88) and slow (p89) migrating forms of phosphorylated STAT3. (C) Preincubation with H7 but not with HA1004 inhibited anti-Ig–induced activation of STAT3. (D and E) Rapamycin inhibited anti-Ig–induced activation of STAT3. Primary B cells (1.5 × 10^7) were incubated in medium alone (−) or were stimulated with F(ab′)2 goat anti-mouse IgM (15 μg/ml; aIg) for the indicated times, after which nuclear extracts were prepared. Before stimulation, some B cell cultures were pretreated for 30 min with CHX (10 μg/ml) as indicated. Nuclear extracts were also analyzed by SDS-PAGE on 7.5% gels followed by immunoblotting with phosphotyrosine705STAT3–specific antibody, detected by ECL. Arrows indicate fast (p88) and slow (p89) migrating forms of phosphorylated STAT3. (D) Rapamycin inhibits anti-Ig–induced activation of STAT3. Primary B cells (1.5 × 10^7) were incubated in medium alone (−) or were stimulated with F(ab′)2 goat anti-mouse IgM (15 μg/ml; aIg) for the indicated times, after which nuclear extracts were prepared. Before stimulation, some B cell cultures were pretreated with CHX (10 μg/ml) as indicated. Nuclear extracts were also analyzed by EMSA as described in Fig. 1 (E). Arrows indicate positions of nucleoprotein complexes containing SIF A, B, and C. (C) Induction of STAT3 after anti-Ig treatment is blocked by cycloheximide. Primary B cells were incubated in medium alone (M) or were treated with IL-6 (1,000 U/ml) for 15 min, or with F(ab′)2 goat anti-mouse IgM (15 μg/ml; aIg) for 3 h, after which nuclear extracts were prepared. Before stimulation, some B nuclear extracts were also analyzed by EMSA as described in Fig. 1 (E). Arrows indicate positions of nucleoprotein complexes containing SIF A, B, and C.
also completely blocked the formation of the SIF A-binding complex in EMSA experiments conducted using nuclear extracts from B-2 cells stimulated with anti-Ig for 3 h (Fig. 4 B). These results suggest that nuclear localization of phosphorysine705STAT3, and the appearance of nuclear SIF A in B-2 cells stimulated by anti-Ig requires serine/threonine phosphorylation and further implicate STAT3 in the composition of the anti-Ig-induced SIF A complex.

The delayed tyrosine phosphorylation of STAT3 after sIg ligation in B-2 cells suggested that the synthesis of an intermediary protein is required for this response. To test this possibility, B-2 cells were stimulated with anti-Ig in the presence of the protein synthesis inhibitor cycloheximide (CHX) and nuclear extracts were prepared. CHX completely blocked the induction of phosphorysine705STAT3 in nuclear extracts from B-2 cells treated with anti-Ig for 3 h, whereas CHX had no effect on phosphorysine705STAT3 stimulated by IL-6 (Fig. 4 C). CHX also abrogated the formation of the SIF A-binding complex in EMSA experiments performed using nuclear extracts from B-2 cells stimulated with anti-Ig for 3 h (data not shown). Thus, de novo protein synthesis is required for induction of both SIF A and of phosphorysine705STAT3 by anti-Ig.

Since anti-Ig is a mitogenic stimulus for B-2 cells, we reasoned that induction of STAT3 via this novel mechanism may be sensitive to immunosuppressive drugs that inhibit B cell proliferation, such as cyclosporin A, FK506, and rapamycin (37, 38). Immunoblot analysis of nuclear extracts from the B-2 cell preparation after treatment with CsA had a minimal effect on nuclear phosphotyrosine705STAT3, whereas CHX had no effect on phosphotyrosine705STAT3 (data not shown). Further, B-2 cells treated with CsA had a minimal effect on nuclear expression of phosphorysine705STAT3, after anti-Ig stimulation but completely inhibited nuclear phosphorysine705STAT3 induced by the combination of PMA and the calcium ionophore, ionomycin (data not shown) demonstrating an additional level of specificity for the effect of rapamycin on anti-Ig-induced STAT3.

Both the delayed appearance and dependence on protein synthesis of phosphorysine705STAT3 in B-2 cells after anti-Ig stimulation raised the possibility that sIg-mediated STAT3 induction may be due to the release of cytokines from the B cells themselves or from other contaminating cells in the B-2 cell preparation after treatment with anti-Ig. To address this question, nuclear extracts from the mature B cell line BAL-17 were prepared and immunoblotted for phosphorysine705STAT3 after stimulation with anti-Ig. Phosphorysine705STAT3 was induced in BAL-17 B cells by anti-Ig treatment with similar kinetics to that observed in B-2 cells (data not shown) ruling out a role for a factor secreted by a contaminating non-B cell. In addition, culture supernatants from B cells stimulated by anti-Ig for 3 h were transferred to naïve cells, from which nuclear extracts were prepared after 15 min and tested for the presence of phosphorysine705STAT3 by immunoblotting. Supernatants from cultures treated with anti-Ig for 3 h did not induce appreciable rapid tyrosine phosphorylation of STAT3 in previously naïve cells (data not shown), as would be expected of a cytokine-mediated response. These results, coupled with the sensitivity of this response to rapamycin, which does not inhibit cytokine-mediated STAT signaling, suggest that the delayed tyrosine phosphorylation of STAT3 is specific to anti-Ig treatment and is not the result of cytokine release or synthesis triggered by cell activation.

Prolonged exposure of B-2 cells to anti-Ig (e.g., for 2.5 d) has been shown to result in the acquisition of surface CD5 expression and proliferative responsiveness to PMA (39, 40). The possibility that prolonged sIg crosslinking produces a B-1–like basal level of nuclear activated STAT3 was tested by treating B-2 cells with anti-Ig for several days before nuclear extraction. Although B-2 cells treated with anti-Ig for 2.5 d responded to PMA by cell cycle progression to S phase, sIg-mediated nuclear SIF A (which was apparent at 3 h) had disappeared by this time (Fig. 5). This result indicates that STAT3 induced by anti-Ig in B-2 cells is only transiently expressed, and thus long term T cell–independent type II (TI-2) antigenic stimulation of B-2 cells does not recapitulate the profile of activated STAT3 characteristic of B-1 cells, despite inducing other B-1–like changes. These results suggest that activated STAT3 expression is an intrinsic and unique characteristic of B-1 cells.
In conclusion, we have identified constitutive nuclear activated STAT3 in normal murine B-1 lymphocytes, representing the first nuclear transcriptional identifier for this developmentally regulated B cell population. The B-1 cell subset has been linked to spontaneously arising B cell tumors, and STAT3 has been found to be activated in a subset has been linked to spontaneously arising B cell tumors, developmentally regulated B cell population. The B-1 cell representing the first nuclear transcriptional identifier for this activated STAT3 in normal murine B-1 lymphocytes, represented B cells, HTLV-I–transformed T cells, and v-src-transformed fibroblasts (17–20). Basal levels of nuclear phosphorylated STAT3 may reflect, or may cause, the activated state of B-1 cells, and may contribute to the self-renewing growth characteristics and the oncogenic potential of normal B-1 cells in vivo. In contrast, bothegr-1 and c-myc mR N A levels do not differ between B-1 and B-2 cells (41).

There has been considerable debate over whether B-1 cells are derived from a separate lineage of progenitor cells or represent B-2 cells that have undergone internal biochemical and external cell surface marker changes due to prior activation or differentiative responses, such as those delivered by TI-2 antigens (42, 43). Our data suggest that one activity of sIg cross-linking in conventional B cells is to activate STAT3, which occurs in delayed fashion, involves the transient induction of predominantly the STAT3 s isoform in anti-Ig-stimulated B-2 cells and suggests that cross-linking sIg alone does not result in similar nuclear expression, in B-2 cells, of this activated transcription factor present in B-1 cells. Therefore, constitutive B-1 cell STAT3 expression suggests that the development of these B cells cannot be explained by TI-2 antigen-mediated influences alone, and that STAT proteins play a role in directing the unique behavioral and phenotypic characteristics of this population of normal cells.

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