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Activation of Jak/STAT proteins involved in signal transduction pathway mediated by receptor for interleukin 2 in malignant T lymphocytes derived from cutaneous anaplastic large T-cell lymphoma and Sezary syndrome

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ABSTRACT **Signaling through the interleukin 2 receptor (IL-2R) involves phosphorylation of several proteins including Jak3, STAT5, and, in preactivated cells, STAT3. In the present study, we examined the functional status of the IL-2R-associated Jak/STAT pathway in malignant T lymphocytes from advanced skin-based lymphomas: anaplastic large T-cell lymphoma (ALCL) and Sezary syndrome (SzS). Proliferation of three ALCL cell lines (PB-1, 2A, and 2B) was partially inhibited by rapamycin, a blocker of some of the signals mediated by IL-2R, but not by cyclosporin A, FK-506, and prednisone, which suppress signals mediated by the T-cell receptor. All the cell lines expressed on their surface the high-affinity IL-2R (α, β, and γc chains). They showed basal, constitutive phosphorylation, and coassociation of Jak3, STAT5, and STAT3. Weak basal phosphorylation of IL-2R γc was also detected. In regard to SzS, peripheral blood mononuclear cells from 10 of 14 patients showed basal phosphorylation of Jak3, accompanied by phosphorylation of STAT5 in 9 patients, and STAT3 in 4 patients. However, *in vitro* overnight culture of SzS cells without exogenous cytokines resulted in markedly decreased Jak3 and STAT5 phosphorylation, which could be reversed by stimulation with IL-2. This indicates that the basal phosphorylation of Jak3 and STAT5 in freshly isolated SzS cells is induced rather than constitutive. The basal activation of the Jak/STAT pathway involved in IL-2R signal transduction in ALCL and SzS cells reported here suggests that this pathway may play a role in the pathogenesis of cutaneous T-cell lymphomas, although the mechanism (induced versus constitutive) may vary between different lymphoma types.**

High-affinity interleukin 2 receptor (IL-2R) is composed of three chains: α which is specific for IL-2, β, and common γ (γc; refs. 1 and 2). The γc is also a component of receptors for several other cytokines (3–5). Together with the same β chain and a different, IL-15-specific α chain, γc forms a fully functional IL-15R (6–9). Finally, γc codimerized with the respective cytokine-specific chains transduces signals mediated by IL-4, IL-7, and IL-9 (10–13). Intact structure and function of the γc is fundamental for the proper maturation and activation of T lymphocytes and other cells of the immune system since mutations of the γc gene lead to the X-linked severe combined immunodeficiency syndrome in both men (1, 14) and mice (15–17). Interaction of the cytokine receptors such as IL-2R with their ligands induces activation of the intracellular tyrosine kinases (2, 18). The cytoplasmic domain of the γc chain is associated with the tyrosine kinase Jak3, whereas the β chain is associated with Jak1 (19, 20). Binding of IL-2 to the IL-2R results in activation of these two kinases and tyrosine phosphorylation of several substrates, including Jak3 and Jak1 themselves, as well as the IL-2R γc and β chains (21, 22). The phosphorylated IL-2R chains recruit proteins such as STAT5 (23–25) and, in phytohemagglutinin (PHA)-preactivated T-cell blasts, STAT3 (24, 26). The STATs, upon phosphorylation, presumably by the Jaks, translocate into the nucleus and bind to DNA to initiate transcription of IL-2 responsive genes. Involvement of Jak3 is crucial for transduction of signals mediated by γc because mutations of Jak3 result in severe immunodeficiency in patients (27, 28) and mice (29, 30), which mimics that seen in mutations of the γc chain (1, 14–17). Some of the signals mediated by the IL-2R can be inhibited by rapamycin (31), which appears to exert this inhibition by interfering with induction of *bcl-2* (32).

Cutaneous T-cell lymphoma (CTCL) is the most frequent lymphoma occurring in the skin (33, 34). At presentation, CTCL is usually an indolent, low-grade tumor composed of atypical T lymphocytes with small to medium-sized hyperchromatic and highly irregular (cerebriform) nuclei (35, 36). CTCL has a tendency to progress over time. When it spreads into the blood, the diagnosis of Sezary syndrome (SzS) is made (37–39). Progression to a high-grade lymphoma (35, 36) composed of cells with large nuclei and sometimes anaplastic features also occurs, leading to the diagnosis of the secondary (anaplastic) large T-cell lymphoma (ALCL).

Because signals mediated through IL-2R are so important in activation of normal T cells, we examined the functional status of IL-2R-associated Jak/STAT signal transduction pathway in malignant T cells from the lymphomas involving skin. Our data demonstrate that this pathway shows basal activation, respectively constitutive and induced, in cells from ALCL and SzS. IL-2R signal transduction pathway may therefore play a role in the pathogenesis of cutaneous T-cell malignancies, although the mechanism of its activation may be different between these lymphoma types.

MATERIALS AND METHODS

Cell Lines. Three cell lines (PB-1, 2A, and 2B), established from a patient with a progressive cutaneous T-cell lymphoproliferative disorder have been described in detail (41, 42). In

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Abbreviations: IL, interleukin; R, receptor; γc, common γ chain; PHA, phytohemagglutinin; SzS, Sezary syndrome; ALCL, anaplastic large T-cell lymphoma; PBMC, peripheral blood mononuclear cells; PE, phycoerythrin; Ab, antibody.

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brief, the PB-1 cell line was obtained at a relatively early stage of the patient's cutaneous T lymphoma from neoplastic T cells circulating in peripheral blood. The 2A and 2B lines were established at a later, aggressive stage from two separate skin nodules, which represented a high-grade, T-cell ALCL. The common clonal origin of all three T-cell lines was demonstrated by cytogenetics and T-cell receptor gene rearrangement (41). The same clone was found in patient's lymphoma tissues. The cell lines were also similar to fresh biopsy specimens in regard to morphology and immunophenotype (42) and retained in culture features of the original lymphoma. LCL is a lymphoblastoid B-cell line obtained from the same patient as the PB-1, 2A, and 2B lines, by *in vitro* infection with Epstein–Barr virus. The SZ-4 line, kindly provided by T. Abrams (Hahnemann University), was derived from a SzS patient and bears close morphological, phenotypic, and genotypic resemblance to the original tumor (43). Growth of the SZ-4 line is IL-2 dependent. The human NK (YT) cell line (44) was kindly provided by J. Yodoi (Kyoto University). All the cell lines were propagated in complete RPMI 1640/10% FBS medium (42).

Patients and Healthy Donors. Fourteen patients with SzS were studied. All had erythroderma, lymphadenopathy, and peripheral blood involvement by morphologic, immunophenotypic, molecular, and/or cytogenetic criteria. The percent of circulating Sezary cells ranged from 1–91% of lymphoid cells (median, 47%) based on morphology and from 42–99% (median, 85%) based on flow-cytometry immunophenotyping ($CD4^+/CD7^-$ T lymphocytes). In all cases a clonal population was detected by T-cell receptor gene rearrangement and/or cytogenetic analysis. Two healthy adults served as normal controls. Peripheral blood mononuclear cells (PBMC) were obtained as described (40) by centrifugation on Ficoll/Paque gradient.

IL-2 and Immunosuppressive Agents. Recombinant human IL-2 was obtained from two sources: R & D Systems, or was kindly provided by C. Reynolds (National Cancer Institute, Frederick, MD). Rapamycin, cyclosporin A, and FK-506 were kindly provided by, respectively, Wyeth–Ayerst, Sandoz, and Fujisawa Pharmaceutical (Osaka). Prednisone was purchased from Sigma.

Antibodies and Peptides. Anti-IL-2Rα and anti-IL-2Rβ monoclonal antibodies (Ab), conjugated with phycoerythrin (PE) were purchased from Becton Dickinson. Polyclonal goat anti-IL-2R γc Ab was kindly provided by M. Tsang (R & D Systems). Rabbit polyclonal Ab against IL-2R γc, Jak1, Jak3, STAT3, and STAT5 and the relevant peptides were purchased from Santa Cruz Biotechnology. Anti-phosphotyrosine 4G10 monoclonal Ab was purchased from Upstate Biotechnology (Lake Placid, NY). PE-conjugated donkey anti-goat and peroxidase conjugated donkey anti-rabbit and anti-mouse Ab were obtained from Jackson ImmunoReseach.

Proliferation Assays. These tests were performed as described (40, 45). In brief, the cell lines or PHA-stimulated PBMC were cultured for 52 hr in triplicate at 2×10^4 cells/well in the presence of various concentrations of the immunosuppressive drugs. After 14-hr pulse with 0.5 μCi of [^{3}H]thymidine (1 Ci = 37 GBq), radioactivity of the cells was measured.

Flow-Cytometry Analysis Flow cytometry for expression of IL-2R α, β, and γc chains was performed by direct and indirect immunostaining as described (40, 45). In brief, cells were stained at 4°C with the appropriate Ab, either PE-conjugated (anti-α and -β) or unconjugated (goat anti-γc) Ab, which was followed by a secondary, PE-conjugated $F(ab')_2$ fragment of the donkey-anti-goat Ab. Cells not treated with the IL-2R chain-specific Ab served as controls.

Protein Phosphorylation and Coprecipitation Assays. Cells ($10–15 \times 10^6$) were exposed to 500U of IL-2 (R & D Systems) or medium alone for 20 min at 37°C, lysed for 20 min in 1 ml ice-cold lysis buffer [0.5% Nonidet P-40/10 mM Tris·HCl, pH 7.4/150 mM NaCl/0.4 mM EDTA/1 mM sodium orthovanadate/0.5 mM phenylmethylsulfonyl fluoride/10 mM NaF/3 μg each of pepstatin, leupeptin, chymostatin, and aprotinin per ml (Sigma)]. The cells were centrifuged at 15,000 rpm and the supernatant was precleared overnight at 4°C with protein A-Sepharose (Sigma). The appropriate Ab was added and incubated for 90 min on ice. In some experiments the Ab was preincubated for 2 hr at room temperature with the relevant, inhibitory peptide. Then the protein A-Sepharose was added to precipitate the immune complexes for 2 hr or overnight at 4°C. The immunoprecipitates were washed, boiled, suspended in reducing SDS loading buffer, separated on a 10% polyacrylamide/SDS gel, and transferred electrophoretically to hybridization transfer membranes. The membranes were blocked with 2% bovine serum albumin in TBST buffer (10 mM Tris·HCl, pH 7.4/75 mM NaCl/1 mM EDTA/0.1% Tween 20) for at least 2 hr at room temperature or overnight in a cold room, incubated with the relevant primary Ab, washed, incubated with peroxidase-conjugated secondary Ab, and washed again. Blots were developed using the ECL chemiluminescence reagents (Amersham).

RESULTS

Effect of Immunosuppressive Drugs on Proliferation of Malignant T-Cell Lymphoma Lines. Activation of normal T lymphocytes occurs in two stages (46). In the first, T cells receive signals through the T-cell receptor complex and receptors for costimulatory molecules. In the second stage, they respond to cytokines such as IL-2. To determine which of these activation pathways is important in the growth of malignant T cells, the three ALCL-derived T-cell lymphoma lines (PB-1, 2A, and 2B) were exposed to several immunosuppressive agents. As shown in Fig. 1, only rapamycin, which inhibits certain signals mediated by the IL-2R (31, 32), partially suppressed proliferation of the cell lines. None of the agents that inhibit, at various levels, signals mediated through the T-cell receptor—cyclosporin A, FK-506 (31), and prednisone (47, 48)—had any effect.

Surface Expression of the IL-2R Complex by Malignant T-Cell Lines. To determine if the IL-2R chains can be detected on the surface of these cell lines and, therefore, make them receptive to IL-2 and, possibly, the other cytokines which use γc, we performed flow cytometric analysis. As shown in Fig. 2, IL-2R chains could be identified on the surface of all three cell lines with the PB-1 line, which was established from a relatively early stage of the lymphoma, showing the highest expression of these chains. As observed previously (42), concentration of the α chain was relatively high (mean channel: 4836, 609, and 948 for PB-1, 2A, and 2B, respectively). As expected, the control NK cell line, YT, displayed relatively low expression of the α chain (mean channel, 218). β chain was expressed by the T-cell lymphoma lines at much lower concentration than the α chain (mean channel: 72, 31, 28, and 313 for PB-1, 2A, 2B, and YT, respectively). Expression of γc was comparable to the expression of the β chain (mean channels, 40, 27, 23, and 67). Thus, the cell lines expressed all the components of the high-affinity IL-2R and, therefore, should be able to respond to this cytokine.

Phosphorylation of Jak and STAT Proteins Involved in IL-2R-Mediated Signal Transduction. To evaluate the activation status of the IL-2R-associated signal transduction pathway in the T-cell lymphoma lines, we examined phosphorylation of Jak1, Jak3, STAT3, and STAT5 in the ALCL-derived T cell lines without and with exposure of the cells to IL-2. A weak band corresponding to phosphorylated Jak1 kinase was detected in all the lines (Fig. 3*A*). Whereas the PB-1 line required stimulation by IL-2 to display Jak1 phosphorylation, 2A and 2B lines showed basal, constitutive Jak1 phosphorylation that was not augmented by addition of IL-2. In contrast to Jak1, phosphorylation of Jak3, STAT3, and STAT5 were easily

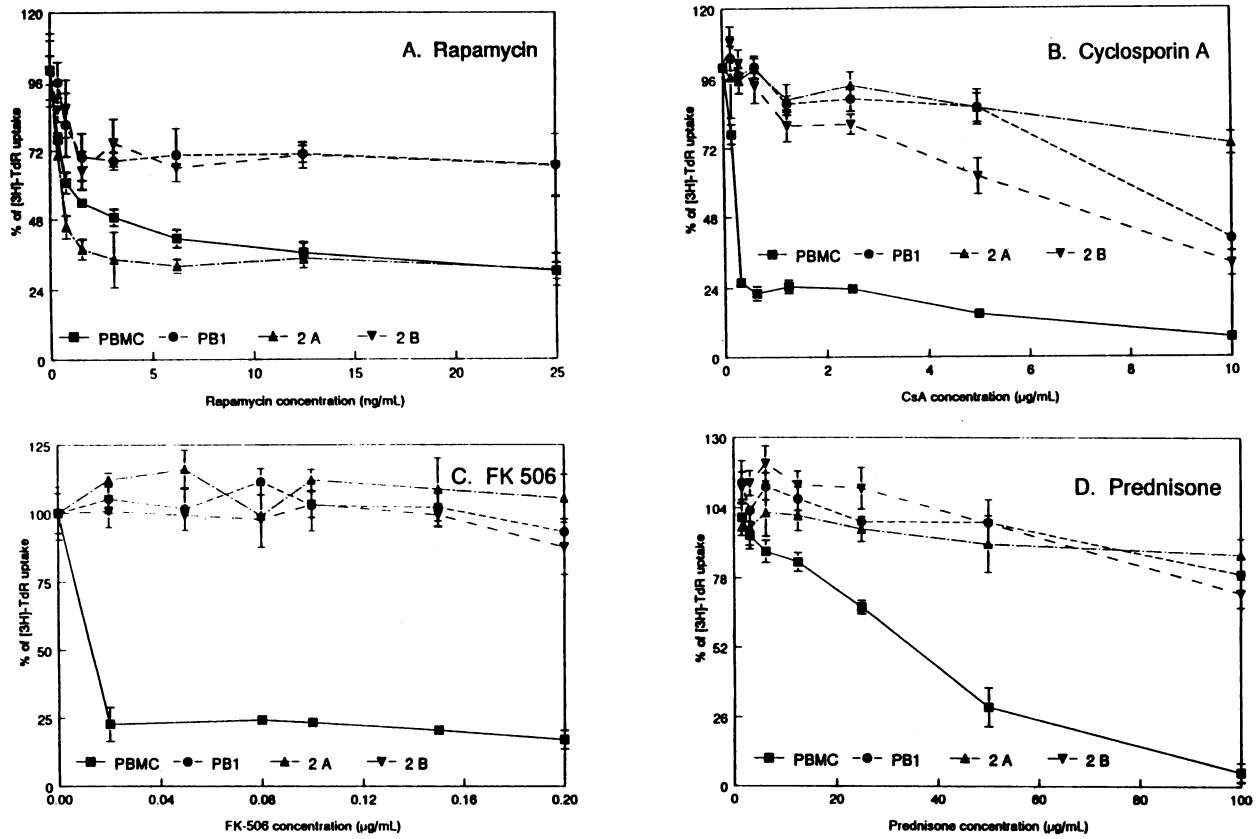

FIG. 1. Inhibition of proliferation of the malignant T-cell lymphoma lines (PB-1, 2A, and 2B) and control PHA-stimulated PBMC by immunosuppressive agents: rapamycin (*A*), cyclosporin A (*B*), FK-506 (*C*), and prednisone (*D*). The results are expressed as a percentage of the proliferative response in the presence of the drugs as compared with the medium alone.

detectable (Fig. 3 *B–D*). The pattern of phosphorylation of these proteins, particularly of Jak3, was similar to the one of Jak1. The PB-1 line required IL-2 for maximal Jak3 phosphorylation, whereas the 2A and 2B lines showed constitutive, maximal phosphorylation, nonresponsive to stimulation with IL-2 (Fig. 3*C*). Basal phosphorylation of STAT3 (Fig. 3*C*) and STAT5 (Fig. 3*D*) appeared maximal in all three lines. The control, Epstein–Barr virus-transformed B-cell LCL line derived from the same patient did not show phosphorylation of any of these Jak and STAT proteins, either basal or after exposure to IL-2 (Fig. 3 *C* and *D*, and data not shown). Because Jak3 associates with, and presumably phosphorylates, IL-2Rγc (19, 20), we also determined the phosphorylation status of the γc in the PB-1 and 2B lines (Fig. 3*E*). As with Jak3, PB-1 showed weak basal phosphorylation of the γc, significantly enhanced by exposure of the cells to IL-2. The 2B line showed

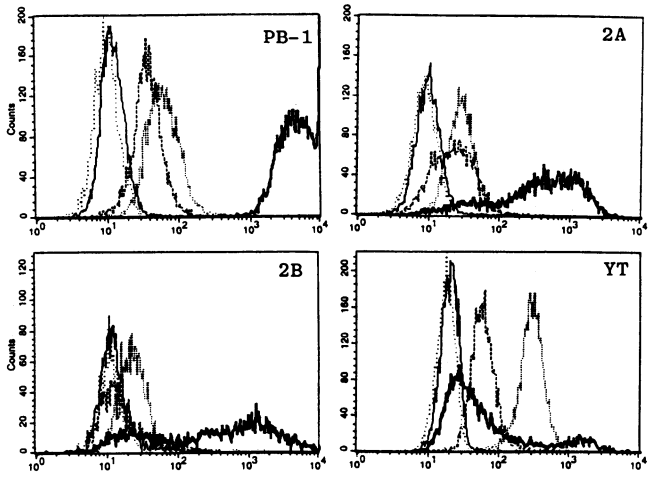

FIG. 2. Cell-surface expression of IL-2R α, β, and γc chains by malignant T-cell lymphoma lines (PB-1, 2A, and 2B) and a control NK-cell line (YT). The cells were stained with the appropriate Ab either PE-conjugated [anti-α (——) and -β (···)] or unconjugated [anti-γc (---)], with the latter followed by a secondary, PE-conjugated Ab. Cells treated with medium (-- -- --) or only the secondary Ab (. . .) served as controls.

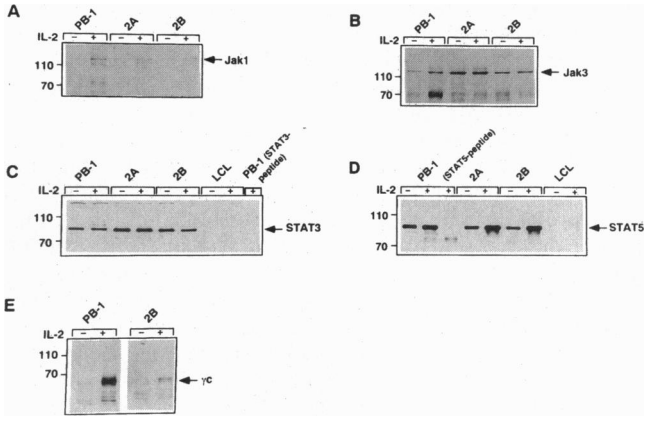

FIG. 3. Phosphorylation of proteins associated with IL-2R signal transduction pathway in the malignant T-cell lines (PB-1, 2A, and 2B) without (−) and with (+) stimulation by IL-2: Jak1 (*A*), Jak3 (*B*), STAT3 (*C*), STAT5 (*D*), and IL-2R γc (*E*). Epstein–Barr virus-transformed B-cell line (LCL) derived from the same patient as the malignant T-cell lines was used as a control. In some experiments (*C*, lane 9 and *D*, lane 3), the primary antibody was preincubated with the relevant, inhibitory peptide to provide an additional control.

a similarly weak band in nonstimulated cells, which was moderately enhanced by stimulation with IL-2.

Associations Among Jak3, STAT5, and STAT3 Proteins. To determine if there were associations between the Jak and STAT proteins involved in IL-2R-mediated signal transduction in the malignant T lymphocytes studied here, we performed several coprecipitation experiments (Fig. 4). Immunoprecipitation with anti-STAT5 antibody, followed by immunoblotting with anti-Jak3 antibody, resulted in visualization of the Jak3 protein (Fig. 4*A*). Jak3 could be detected in the PB-1, 2A, and 2B lines in both IL-2 nonexposed and exposed cells. This finding documents constitutive association between Jak3 and STAT5 in these ALCL-derived T cells. Similar constitutive association was found in between Jak3 and STAT3 (Fig. 4*B*) and STAT5 and STAT3 (Fig. 4*C*).

Phosphorylation of IL-2R-Associated Jak/STAT Proteins in Freshly Isolated Cells from Patients with SzS. To determine if activation of the IL-2R-associated signal transduction pathway observed in the ALCL-derived malignant T cells is also present in SzS cells, we tested phosphorylation of Jak3, STAT3, and STAT5 proteins in freshly isolated PBMC from 14 patients with SzS. Results from one, representative patient (patient 2) are shown in Fig. 5*A*, and the data from all patients are summarized in Table 1. As can be seen in Table 1, PBMC from 10 of 14 patients showed phosphorylation of Jak3 without exposure to IL-2. Stimulation by IL-2 either markedly enhanced this basal Jak3 phosphorylation or induced it in the 4 patients who were negative without IL-2 stimulation. Nine of the 10 patients who displayed the basal Jak3 phosphorylation also showed basal, frequently weak, phosphorylation of STAT5, and 4 showed phosphorylation of STAT3. Similar to Jak3 phosphorylation, IL-2 usually had an augmenting and/or inducing effect on STAT3 and STAT5 phosphorylation. As

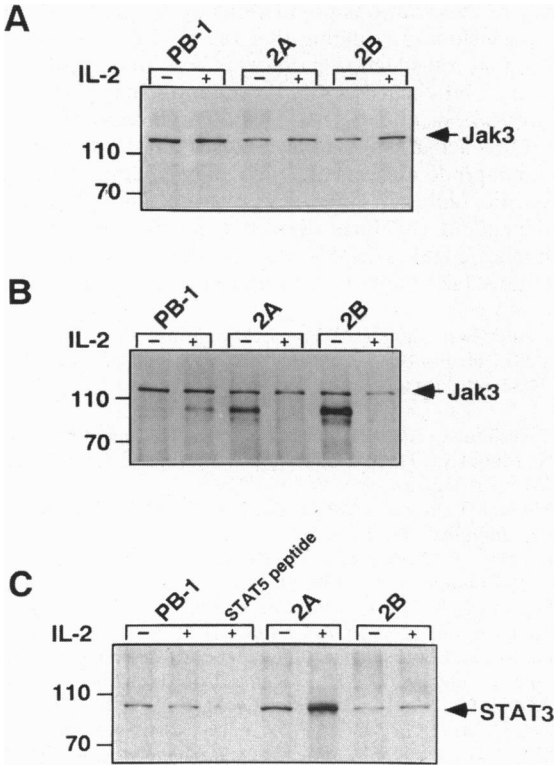

FIG. 4. Association of Jak3, STAT5, and STAT3 proteins. (*A*) STAT5 immunoprecipitation/Jak3 immunoblotting. (*B*) STAT3 immunoprecipitation/Jak3 immunoblotting. (*C*) STAT5 immunoprecipitation/STAT3 immunoblotting. In one of the experiments (*C*, lane 3), the anti-STAT5 Ab was preincubated with the inhibitory STAT5 peptide to provide an additional control.

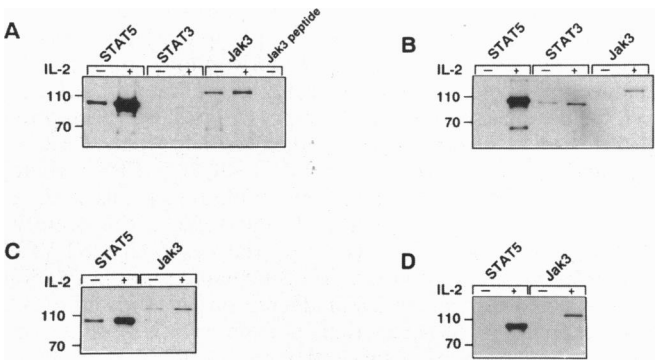

FIG. 5. Jak/STAT phosphorylation in PBMC from a patient with SzS and in a SzS-derived cell line (SZ-4). (*A*) Phosphorylation of freshly isolated patient's cells. (*B*) Phosphorylation of patient's cells after an overnight culture. (*C*) Phosphorylation in SZ-4 cells precultured in IL-2. (*D*) Phosphorylation in SZ-4 cells precultured without IL-2.

expected, the control, normal PBMC and PHA-stimulated blasts required IL-2 stimulation to phosphorylate, respectively, Jak3 and STAT5, and Jak3, STAT5, and STAT3 proteins.

Phosphorylation of IL-2R-Associated Jak/STAT Proteins in Short-Term Cultured Cells from SzS Patients. There are at least two possible, alternative mechanisms responsible for the

Table 1. Phosphorylation of IL-2R-associated proteins in freshly isolated and cultured SzS cells

	Jak3		STAT3		STAT5	
IL-2*	−	+	−	+	−	+
Freshly isolated cells						
Patient no.						
1	−	+	−	−	−	+
2	+	++	−	+/−	+	++
3	−	+	−	−	+/−	+
4	−	+	NT	NT	−	+
5	−	+	−	−	−	+
6	−	+	−	+	+/−	+
7	+	NT	−	NT	+/−	NT
8	+	NT	−	NT	−	NT
9	+	NT	−	NT	−	NT
10	+	++	+/−	NT	+/−	++
11	+	NT	+/−	NT	+/−	NT
12	+	NT	+	NT	+/−	NT
13	+	NT	+	NT	+/−	+
14	+	NT	−	NT	+/−	++
PBMC†	−	+	−	−	−	+
PHA-blasts‡	−	+	−	+	−	+
Cultured cells						
Patient§ no.						
2	−	++	+/−	+	+/−	+
10	+/−	+	NT	NT	−	++
13	−	+	NT	NT	+/−	++
14	−	+	NT	NT	−	++
SZ-4 cell line						
IL-2 cultured	+/−	+	NT	NT	+	++
IL-2 free¶	−	+	−	NT	−	++

NT, not tested; −, no band visible; +/−, weak band; +, strong band; ++, very strong band.
*Cells were exposed *in vitro* to 500 units of IL-2.
†PBMC from a healthy donor.
‡Normal PBMC tested after 5 days of stimulation with PHA.
§PBMC from SzS patients were preincubated for 14 hrs in RPMI 1640/10% FBS medium.
¶Cells from the IL-2 dependent, SzS patient-derived SZ-4 cell line were preincubated without IL-2 for 14 hr in RPMI 1640/10% FBS medium.

observed basal Jak3/STAT3/STAT5 phosphorylation in the cells from SzS patients. First, this phosphorylation may be constitutive as seen in the ALCL-derived lines, or it may be induced *in vivo*, presumably by cytokine(s) that act via IL-2R γc. To distinguish between these possibilities, we precultured PBMC isolated from four selected SzS patients (patients 2, 10, 13, and 14) for 12–16 hr in RPMI 1640/10% FBS medium without addition of any exogenous cytokines such as IL-2. As can be seen in Fig. 5*B* (patient 2) and Table 1, this preincubation usually markedly decreased the basal Jak3/STAT3/STAT5 phosphorylation. The IL-2-mediated phosphorylation was affected to a much lesser degree or not affected at all. These findings indicate that phosphorylation of signal-transducing proteins associated with IL-2R γc is probably induced in Sezary cells *in vivo* rather than being constitutive. To provide additional evidence to support this conclusion, we assessed phosphorylation of the Jak/STAT proteins in SZ-4 T-cell line derived from a patient with SzS. Growth of the SZ-4 cells is, in our hands, strictly IL-2 dependent; withdrawal of this cytokine leads to a resting phase that can be reversed by addition of IL-2 (M.A.W., unpublished observation). As shown in Fig. 5*C* and Table 1, the pattern of phosphorylation of Jak3 and STAT5 in SZ-4 cells cultured for 2–3 days in IL-2 was very similar to the one seen in freshly isolated cells from patients with SzS (Fig. 5*A* and Table 1): both Jak3 and STAT5 were phosphorylated and this phosphorylation was markedly enhanced by stimulation with IL-2. As was the case with the short-term cultured SzS cells (Fig. 5*B* and Table 1), overnight preincubation of SZ-4 cells in medium without IL-2 virtually eliminated the basal Jak3/STAT5 phosphorylation but did not affect phosphorylation of these proteins in response to IL-2 (Fig. 5*D* and Table 1).

DISCUSSION

In this study we examined the functional status of Jaks and STATs involved in the IL-2R signal transduction pathway in malignant T cells from advanced lymphomas involving skin. All three cell lines derived from a single patient with a secondary cutaneous ALCL showed constitutive activation of the Jak/STAT pathway as documented by basal phosphorylation and coassociation of these proteins. Freshly isolated cells from 10 of 14 patients with SzS also showed phosphorylation of Jak3, usually accompanied by phosphorylation of STAT5 and, less frequently, STAT3. However, an overnight *in vitro* culture of the SzS-derived cells resulted in a marked decrease in Jak3 and STAT5 phosphorylation. This indicates that the basal activation of the Jak/STAT proteins in SzS cells is, in contrast to the ALCL cell lines, induced and not constitutive.

Several observations indicate that this discrepancy represents a biologic difference between SzS and ALCL rather than a variation between fresh and cultured cells. First, secondary ALCL represents an aggressive, high-grade lymphoma composed of large, highly atypical cells with highly abnormal immunophenotype and karyotype (35, 36, 49). SzS, however, is usually a low-grade lymphoma with a more prolonged course, less atypical, small to medium-sized cells, and generally less complex, immunophenotype, and karyotype (35, 36, 49). Also, the ALCL-derived PB-1, 2A, and 2B T-cell lines are virtually identical to the original lymphoma cells as determined by morphologic, immunologic, cytogenetic, and molecular criteria (41, 42). The cell lines are also very stable in culture as shown by the repeated immuno- and cytogenetic phenotyping. Furthermore, the PB-1 cell line, which was derived from a clinically less-aggressive stage of the patient's disease displayed only a partial basal activation of the Jak3, which could be augmented by IL-2, whereas the 2A and 2B lines, from a highly aggressive stage of the same lymphoma, showed maximal basal activation of Jak3, nonresponsive to IL-2. Thus, PB-1 may represent an "intermediate" activation status of the IL-2R related Jak/STAT pathway, which is consistent with the other features of this line. Finally, the SzS-derived SZ-4 cell line required IL-2 for proliferation and activation of Jak3 and Stat 5 proteins similar to the freshly isolated SzS cells.

The dependence of SzS-derived lines on exogenous cytokines has also been noted by others (50). It appears, therefore, that low-grade T-cell lymphomas of the skin may still be dependent on autocrine or paracrine cytokines for their growth, whereas the high-grade tumors may become progressively independent of these factors. Evidence that SzS cells appear to produce cytokines of the Th2 type such as IL-4 (51), and that ALCL cells do not produce IL-4 or IL-2 (42, 52, 53), is consistent with such an explanation. Furthermore, the 2A and 2B cell lines studied here express less IL-2R than the PB-1 line (Fig. 2 and ref. 42), despite the fact that they show strong basal phosphorylation of the Jak/STAT proteins (Fig. 3). This finding also argues against an autocrine cytokine that utilizes the IL-2R or its components (e.g., γc) being responsible for the constitutive phosphorylation of the Jak/STAT proteins in the more advanced tumors. Similar constitutive activation of Jak/STAT proteins involved in IL-2R-mediated signal transduction was reported recently in human T-lymphotropic virus, type I (HTLV-I)-positive, transformed T-cell lines (54). Although HTLV-I, and particularly the tax protein, has T-cell activating properties (55), its role in Jak/STAT phosphorylation in these lines remains to be examined. HTLV-I is not responsible for the constitutive Jak/STAT phosphorylation in the ALCL cells we have studied, since PB-1, 2A, and 2B cells do not contain the HTLV-I DNA (M. Wasik, unpublished data) and there is currently no evidence for association between ALCL and HTLV-I. Clearly, further studies, such as search for potentially oncogenic mutation(s) of the proteins involved in the IL-2R-mediated signal transduction are needed to understand the nature of the basal, constitutive activation of the Jak/STAT pathway in these various populations of neoplastic T cells.

In conclusion, our finding that the IL-2R-associated Jak/STAT signal transduction pathway is activated in ALCL and SzS-derived cells suggests that this pathway may play a role in the pathogenesis of cutanous T-cell lymphomas. The mechanism of this activation—induced versus constitutive—may be lymphoma-grade dependent. The recent finding that a novel, Jak-specific inhibitor suppresses growth of Jak2-phosphorylation dependent cell lines derived from patients with acute lymphoblastic leukemia (56) suggests that a similar approach that targets Jak3 might be considered in SzS and ALCL.

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