Distinct Roles for STAT1, STAT3, and STAT5 in Differentiation Gene Induction and Apoptosis Inhibition by Interleukin-9*

(Received for publication, October 21, 1998, and in revised form, June 22, 1999)

Jean-Baptiste Demoulin‡§, Emiel Van Roost‡, Monique Stevens‡, Bernd Groner¶, and Jean-Christophe Renaud¶

From the §Ludwig Institute for Cancer Research and the Experimental Medicine Unit, Université Catholique de Louvain, avenue Hippocrate, 74, B-1200 Brussels, Belgium and the ¶Institute for Experimental Cancer Research, Tumor Biology Center, Breisacher Strasse 117, D-79106 Freiburg, Germany

Interleukin-9 (IL-9) activates three distinct STAT proteins: STAT1, STAT3, and STAT5. This process depends on one tyrosine of the IL-9 receptor, which is necessary for proliferation, gene induction, and inhibition of apoptosis induced by glucocorticoids. By introduction of point mutations in amino acids surrounding this tyrosine, we obtained receptors that activated either STAT5 alone or both STAT1 and STAT3, thus permitting us with the possibility to study the respective roles of these factors in the biological activities of IL-9. Both mutant receptors were able to prevent apoptosis, but only the mutant that activated STAT1 and STAT3 was able to support induction of granzyme A and L-selectin. In line with these results, constitutively activated STAT5 blocked glucocorticoid-induced apoptosis. In Ba/F3 cells, significant proliferation and pim-1 induction were observed with both STAT-restricted mutants, though proliferation was lower than with the wild-type receptor. These results suggest that survival and cell growth are redundantly controlled by multiple STAT factors, whereas differentiation gene induction is more specifically correlated with individual STAT activation by IL-9.

Interleukin-9 (IL-9) is a pleiotropic cytokine that is active on hematopoietic progenitors, lymphocytes, and mast cells (1, 2) and whose excessive expression has been linked to asthma and hematopoietic development (1, 3–6). In particular, IL-9 inhibits proliferation and whose excessive expression has been linked to asthma and hematopoietic progenitors, lymphocytes, and mast cells (1, 2) genes, which are not regulated by IL-2.

genes, such as Ly-6A/E, granzymes, and mast cell-specific IL-15 (10), share some activities with IL-9 such as T cell pro-

liferation in vitro and induction of glucocorticoid-induced apoptosis. Yet, IL-9 specifically induces the expression of several genes, such as Ly-6A/E, granzymes, and mast cell-specific genes, which are not regulated by IL-2.

We recently showed that these effects are mediated by the JAK (Janus kinase)-STAT (signal transducer and activator of transcription) pathway (11). IL-9 activates two JAK tyrosine kinases, JAK1 and JAK3, pre-associated with IL-9R and the IL-2 receptor γ-chain, respectively (11–13). These kinases are responsible for the activation of STAT1, STAT3, and STAT5 transcription factors by IL-9 (11, 14).

Unlike classical kinase substrates, the type of STAT activated by a given cytokine is not dependent on the type of activated JAK kinase, but on the ability of receptor phosphotyrosine motifs to interact with certain STAT SH2 (Src homology 2) domains (15). For instance, the phospho-Tyr-Glu-XX-His sequence in the IFN-γ receptor interacts specifically with STAT1 (16), and phospho-Tyr-XX-Gln motifs in gp130 do so with STAT3 (17). A second factor controlling specificity lies in the DNA sequences recognized by each STAT complex, as shown by EMSA in vitro analysis (18, 19). For example, STAT5 binds to the β-casein promoter element (βCAS) required for the control of this gene by prolactin (20). Nevertheless, other DNA sequences, such as GRR, a Fcγ receptor type I promoter motif responsible for regulation by IFN-γ, bind to most STAT factors (11, 21). However, it is not known if the Fcγ receptor type I gene can be regulated in vivo via all these STAT proteins. More generally, specificity in the regulation of gene expression by different STAT proteins has been studied only to a limited extent (22).

Activation of STAT1, STAT3, and STAT5 by IL-9 depends on a single phosphotyrosine at position 367 in IL-9R. This amino acid is also required for gene induction, cell growth regulation, and apoptosis inhibition by IL-9 (11, 14). The aim of this work was to discriminate between the respective roles of each STAT in IL-9 signaling by mutating amino acids surrounding IL-9R tyrosine 367. Our data point to a specific role for STAT1 and STAT3 in differentiation gene induction. By contrast, protection against apoptosis and proliferation was observed in mutants activating either STAT5 or both STAT1 and STAT3, suggesting redundancy in these cases.

EXPERIMENTAL PROCEDURES

Cytokines—IL-3 was produced by transfected Chinese hamster ovary cells (provided by A. Burgess, Ludwig Institute, Melbourne, Australia). Recombinant human IL-9 and mouse IL-9 and IL-6 were produced in the baculovirus system and purified as described previously (11). Recombinant mouse IFN-γ was provided by W. Fiers (University of Gent, Gent, Belgium).

Plasmid Constructions, DNA Transfection, and Analysis of Transfected Cells—Mutagenesis was performed using the Chameleon double-stranded site-directed mutagenesis kit (Stratagene). Wild-type and mutant hIL-9 cDNAs, as well as the STAT5 5′-VP16-JAK2 construct, were inserted into the pEF-BOS-puro plasmid, which contains a puromycin resistance gene (11, 23).

Murine T lymphoma BW5147 and pro-B Ba/F3 cells were cultured and transfected by electroporation as described (11, 24). After selection of clones with puromycin (1.5 and 3 μg/ml for BW5147 and Ba/F3 cells,
respective), hIL-9R expression was checked by FACS analysis of cells stained with biotinylated anti-hIL-9R antibody A9HR1 (11), followed by phycoerythrin-conjugated streptavidin (Becton Dickinson). Three independent clones expressing a similar level of hIL-9R were selected in each transfection and analyzed in all subsequent experiments.

Propidium iodide staining and hexosaminidase-assays were performed as described previously (7). Ly-6A/E induction was monitored after a 24-h stimulation by FACS analysis. Cells were stained with biotinylated anti-Ly-6A/E antibodies (1 μg/ml; clone D7, Pharmingen, San Diego, CA), followed by phycoerythrin-conjugated streptavidin. L-selectin induction was also analyzed by FACS using the MEL-14 antibody (a gift of A. Van Halteren, Free University of Amsterdam) and fluorescein-conjugated goat anti-mouse immunoglobulin antibody (Becton Dickinson). Granzyme A protease activity was analyzed as described, using fluorescein-conjugated goat anti-mouse immunoglobulin antibody (Becton Dickinson) and phycoerythrin-coupled streptavidin (Becton Dickinson). Three independent experiments were performed as described previously (11).

N-Benzoyloxycarbonyl-L-lysine thiobenzyl ester as a substrate (25).

**Northern Blot Analysis**—RNA was extracted from 10^6 cells using 1 ml of Trizol solution (Life Technologies, Inc.) following the manufacturer’s instructions. Northern blot analysis was performed as described with 10 μg of RNA (25). cDNA probes coding for chicken β-actin, Pim-1, and mouse granzyme A were retrieved from plasmid DNA (25) and 32P-labeled with the Rediprime kit (Amersham Pharmacia Biotech).

**STAT Oligofishing and EMSA**—These experiments were performed as described previously (11). Briefly, nuclear extracts from 10^6 cytokerine-treated cells were mixed with biotinylated GRR oligonucleotide immobilized on agarose-linked streptavidin (GRR Fcy receptor type 1 gene promoter: upper strand, 5′-CCTTTTCTGGGAAATAC-3′; and lower strand, 5′-GGTTCTCTGGGAATAC-3′). After extensive washing, proteins were eluted from the beads and analyzed by Western blotting with the following specific antibody: anti-STAT1 or anti-STAT5 (1 μg/ml; sc591 and sc1081, Santa Cruz Biotechnology) or anti-STAT3 (1 μg/ml; sc698, Santa Cruz Biotechnology). The GRR oligonucleotide was also used for EMSA, in addition to two GAS oligonucleotides derived from the Ly-6A/E gene promoter (upper strand, 5′-TCATATCTGCTGAAGTG-3′; and lower strand, 5′-GGTTCTTTCTGGGAATAC-3′). After extensive washing, proteins were eluted from the beads and analyzed by Western blotting with the following specific antibody: anti-STAT1 or anti-STAT5 (1 μg/ml; sc591 and sc1081, Santa Cruz Biotechnology) or anti-STAT3 (1 μg/ml; sc698, Santa Cruz Biotechnology). The GRR oligonucleotide was also used for EMSA, in addition to two GAS oligonucleotides derived from the Ly-6A/E gene promoter (upper strand, 5′-TCATATCTGCTGAAGTG-3′; and lower strand, 5′-GGTTCTTTCTGGGAATAC-3′). After extensive washing, proteins were eluted from the beads and analyzed by Western blotting with the following specific antibody: anti-STAT1 or anti-STAT5 (1 μg/ml; sc591 and sc1081, Santa Cruz Biotechnology) or anti-STAT3 (1 μg/ml; sc698, Santa Cruz Biotechnology). The GRR oligonucleotide was also used for EMSA, in addition to two GAS oligonucleotides derived from the Ly-6A/E gene promoter (upper strand, 5′-TCATATCTGCTGAAGTG-3′; and lower strand, 5′-GGTTCTTTCTGGGAATAC-3′). After extensive washing, proteins were eluted from the beads and analyzed by Western blotting with the following specific antibody: anti-STAT1 or anti-STAT5 (1 μg/ml; sc591 and sc1081, Santa Cruz Biotechnology) or anti-STAT3 (1 μg/ml; sc698, Santa Cruz Biotechnology). The GRR oligonucleotide was also used for EMSA, in addition to two GAS oligonucleotides derived from the Ly-6A/E gene promoter (upper strand, 5′-TCATATCTGCTGAAGTG-3′; and lower strand, 5′-GGTTCTTTCTGGGAATAC-3′). After extensive washing, proteins were eluted from the beads and analyzed by Western blotting with the following specific antibody: anti-STAT1 or anti-STAT5 (1 μg/ml; sc591 and sc1081, Santa Cruz Biotechnology) or anti-STAT3 (1 μg/ml; sc698, Santa Cruz Biotechnology). The GRR oligonucleotide was also used for EMSA, in addition to two GAS oligonucleotides derived from the Ly-6A/E gene promoter (upper strand, 5′-TCATATCTGCTGAAGTG-3′; and lower strand, 5′-GGTTCTTTCTGGGAATAC-3′). After extensive washing, proteins were eluted from the beads and analyzed by Western blotting with the following specific antibody: anti-STAT1 or anti-STAT5 (1 μg/ml; sc591 and sc1081, Santa Cruz Biotechnology) or anti-STAT3 (1 μg/ml; sc698, Santa Cruz Biotechnology). The GRR oligonucleotide was also used for EMSA, in addition to two GAS oligonucleotides derived from the Ly-6A/E gene promoter (upper strand, 5′-TCATATCTGCTGAAGTG-3′; and lower strand, 5′-GGTTCTTTCTGGGAATAC-3′). After extensive washing, proteins were eluted from the beads and analyzed by Western blotting with the following specific antibody: anti-STAT1 or anti-STAT5 (1 μg/ml; sc591 and sc1081, Santa Cruz Biotechnology) or anti-STAT3 (1 μg/ml; sc698, Santa Cruz Biotechnology).
lication of STAT3 and STAT1 was abolished by the replacement of glutamine 370 with leucine (mut7), and this mutant activated only STAT5. Mutation of proline 369 (mut9) only partially inhibited STAT1 activation. We obtained similar results with transfected Ba/F3 cells (data not shown). Mutations did not significantly modify phosphorylation of tyrosine 367 (Fig. 3C), which is the only IL-9R phosphorylated tyrosine (11), ruling out the possibility that our observations result from differences in IL-9R tyrosine phosphorylation.

Differentiation Gene Induction Correlates with STAT1 and STAT3 Activation—We took advantage of the mut6 and mut7 receptor mutants to define further the role of STAT proteins in the induction of granzyme A expression by IL-9 (11). BW5147 cells were stimulated with hIL-9 (500 units/ml) for 48 h. A, RNA was extracted and analyzed by Northern blotting with a granzyme A or β-actin probe. B, cells were lysed, and granzyme A activity was measured as described (25). To compare independent experiments, the basal activity in untreated BW-hIL-9Rwt cells was arbitrarily defined as 1 unit. The means ± S.E. of three independent experiments are shown. wt, wild-type.

Fig. 4. STAT activation and granzyme A induction by IFN-γ and IL-6. A, BW5147 cells were stimulated with IFN-γ (1000 units/ml) or IL-6 (5 × 10^4 units/ml) for 30 or 15 min, respectively. Nuclear extracts were prepared and incubated with immobilized GRR oligonucleotide. Bound proteins were analyzed by Western blotting with anti-STAT1 or anti-STAT3 antibody. No signal was observed with anti-STAT5 antibody (data not shown). B, cells were stimulated with the same cytokine concentrations for 24 h. RNA was extracted and analyzed by Northern blotting with a granzyme A or β-actin probe.

Role of STAT Proteins in IL-9 Signaling

Fig. 2. STAT activation by wild-type and mutant hIL-9 receptors. A, BW5147 clones expressing similar levels of the indicated receptor were stimulated with hIL-9 (500 units/ml) for 30 min or left untreated. Nuclear extracts were prepared and incubated with biotinylated GRR oligonucleotide immobilized on streptavidin-agarose. Bound proteins were separated by SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting with specific anti-STAT antibodies. Depicted sequences denote amino acids 367–370 of the corresponding hIL-9R (single-letter code). Similar results were obtained with three independent clones.

B, nuclear extracts from transfected BW5147 cells stimulated for 10 min with the indicated cytokines were mixed with labeled GRR oligonucleotide for EMSA analysis. When indicated, supershifts were induced with anti-STAT1, anti-STAT3, or anti-STAT5 antibody. C, BW5147 cells were treated with hIL-9 for 10 min or left untreated. Tyrosine-phosphorylated proteins were immunoprecipitated (IP) from cell lysates with anti-phosphotyrosine antibody 4G10 and analyzed by Western blotting with anti-hIL-9R antibodies. wt, wild-type.
IL-9 activities. We started our study by analyzing the expression of granzyme A, a typical differentiation gene induced by IL-9 in T cell clones and T lymphomas such as BW5147 (25). Granzyme A expression was analyzed by Northern blotting and by a specific protease activity assay (Fig. 3). It was not enhanced by hIL-9 in BW-mut1 cells, showing that this IL-9 activity is STAT-dependent. Granzyme A was induced in cells transfected with the STAT1/STAT3-activating mutant mut6, but not with mut7. This indicated that STAT5 was unable to mediate this effect, which required STAT1 and/or STAT3 activation. In line with this observation, IL-6, which activated STAT3 and, marginally, STAT1, similarly induced granzyme A expression (Fig. 4). Moreover, IFN-γ, which activated only STAT1 in BW5147 cells, had a similar effect, indicating that STAT1 activation is sufficient (Fig. 4).

We obtained similar results for the induction of two other differentiation markers, Ly-6A/E and L-selectin. Expression of Ly-6A/E surface antigen was enhanced 3-fold in BW5147 cells when murine IL-9 was added to the culture medium, as shown by FACS analysis (Fig. 5 A). hIL-9 induced Ly-6A/E in cells transfected with wild-type hIL-9R or mut6, but not with mut1 or mut7 (Fig. 5 A). This effect was also observed with IFN-γ and, to a lesser extent, with IL-6 (32). In Ba/F3 cells, analysis of a number of cell-surface markers revealed that IL-9 (but not IL-3) up-regulated the expression of L-selectin, an adhesion molecule for leukocytes. Like granzyme A and Ly-6A/E, L-selectin was enhanced in Ba/F3-mut6 cells, but not in Ba/F3-mut1 or Ba/F3-mut7 cells (Fig. 5B).

Ly-6A/E induction by IL-9, IL-6, or IFN-γ involves a GAS that is located 1230 base pairs before the first exon of the Ly-6A/E gene (32, 33). Gel shift assays performed with this GAS probe derived from the Ly-6A/E promoter (32). Supershifts were carried out as described in the legend to Fig. 2.

We obtained similar results for the induction of two other differentiation markers, Ly-6A/E and L-selectin. Expression of Ly-6A/E surface antigen was enhanced 3-fold in BW5147 cells when murine IL-9 was added to the culture medium, as shown by FACS analysis (Fig. 5 A). hIL-9 induced Ly-6A/E in cells transfected with wild-type hIL-9R or mut6, but not with mut1 or mut7 (Fig. 5 A). This effect was also observed with IFN-γ and, to a lesser extent, with IL-6 (32). In Ba/F3 cells, analysis of a number of cell-surface markers revealed that IL-9 (but not IL-3) up-regulated the expression of L-selectin, an adhesion molecule for leukocytes. Like granzyme A and Ly-6A/E, L-selectin was enhanced in Ba/F3-mut6 cells, but not in Ba/F3-mut1 or Ba/F3-mut7 cells (Fig. 5B).
activates only STAT5 in these cells, did not induce any complex with the GAS probe (Fig. 6, last lane), although a strong band shift was observed with the GRR probe (data not shown). These observations demonstrated that STAT5 is not able to bind to the GAS element, in contrast to STAT1 and STAT3. Thus, differences in the DNA-binding properties of STAT proteins account for the specific induction of Ly-6A/E by STAT1 and STAT3. It is likely that the L-selectin and granzyme A genes are regulated by STAT factors in a similar way, but the presence of GAS-type sites in their promoters has still to be confirmed.

**Role of STAT in Proliferation and pim-1 Oncogene Induction by IL-9—**We have described that the mut1 mutation abolished IL-9-mediated proliferation of the pro-B Ba/F3 cell line transfected with IL-9R (11). Ba/F3-mut6 and Ba/F3-mut7 clones still proliferated in response to hIL-9, but with a reduced sensitivity and, at least for mut6, to a lesser extent (Fig. 7). Half-maximal proliferation was indeed obtained with 2.2 ± 0.8, 13.3 ± 4.8, and 8.4 ± 1.5 units/ml IL-9 for the wild-type, mut6, and mut7 receptors, respectively. We assume that several STAT-regulated genes might be involved in proliferation, some being specifically activated by STAT5 or STAT1/STAT3 and others being redundantly activated by these transcription factors. The pim-1 proto-oncogene might be a potential candidate since STAT5 has been implicated in its induction by IL-3 in Ba/F3 cells (34). Fig. 8A shows that the amount of pim-1 RNA increased rapidly upon IL-9 treatment. This was not observed in Ba/F3-mut1 cells, in contrast to mut6- or mut7-transfected cells. Although pim-1 induction is slightly more important with the mut6 mutant, our result suggested that this process could be mediated either by STAT5 or by STAT1/STAT3 in response to IL-9. IFN-γ has been shown to induce pim-1 via the binding of STAT1 to a GAS located in the pim-1 promoter (26). Using this sequence in an EMSA experiment, we observed a gel shift in response to IL-9 either in mut6- or mut7-transfected Ba/F3 cells, but not in cells expressing mut1, matching the pim-1 expression pattern (Fig. 8B).

**Protection against Apoptosis Can Be Mediated by Either STAT5 or STAT3 Activation—**IL-9 has been shown to protect T cells against glucocorticoid-induced apoptosis through a STAT-dependent mechanism (11, 14). Dexamethasone-treated BW5147 cells present typical apoptosis features, including DNA fragmentation (7). Here, we used a propidium iodide exclusion assay to quantitatively assess cell death (7). BW5147 cells transfected with wild-type, mut6, or mut7 hIL-9R were fully protected against cell death when treated with IL-9, in contrast to mut1-transfected cells (Fig. 9), suggesting a redundant activity of STAT proteins in this model. To further demonstrate that STAT factors can inhibit dexamethasone-induced apoptosis, we transfected BW5147 cells with a constitutively activated STAT5 construct consisting of a cDNA fusion protein composed of the JAK2 kinase domain and STAT5 whose weak transactivation domain has been replaced by a VP16 domain. This molecule has been shown to selectively transactivate STAT5-sensitive promoters (35). As shown in Fig. 10, BW5147 transfectants expressing this fusion protein had a constitutive STAT DNA-binding activity in nuclear extracts and were more resistant to dexamethasone, demonstrating that STAT5 activation suffices to mediate this effect. By contrast, IFN-γ, which activated STAT1 and induced both Ly-6A/E and granzyme A in BW5147 cells, did not inhibit the effect of dexamethasone, suggesting that STAT1 was not sufficient. IL-6, which weakly activated STAT3, partially inhibited the effect of dexamethasone (Fig. 9) (7). Altogether, these data suggested that anti-apoptotic activity could be mediated by either activated STAT3 or STAT5.

**DISCUSSION**

Activation of STAT1, STAT3, and STAT5 by IL-9 depends on a single phosphorylated tyrosine of IL-9R (tyrosine 367). Here, we show that distinct amino acids surrounding tyrosine 367 are involved in the activation of these factors: full STAT1 activation required proline 369 and glutamine 370; STAT3 required

---

**Fig. 8. pim-1 proto-oncogene expression.** A, transfected Ba/F3 cells were starved for 8 h in cytokine-free and serum-free medium before stimulation with IL-3 (500 units/ml) or hIL-9 (500 units/ml) for 2 h. RNA was extracted, and Northern blotting was performed as described under “Experimental Procedures” with the whole Pim-1 cDNA or a β-actin cDNA as a probe. This experiment was reproduced twice with independent clones. B, EMSAs were performed as described in the legend to Fig. 6 with a pim-1 promoter GAS oligonucleotide, and nuclear extracts from transfected Ba/F3 cells were stimulated as described for A for 30 min (26). wt, wild-type.

**Fig. 9. The mut6 or mut7 receptor confers full protection by hIL-9 against dexamethasone-induced apoptosis.** Transfected BW5147 cells were incubated for 24 h in the presence of dexamethasone (100 ng/ml) and cyclosporin A (500 ng/ml) with or without cytokine (200 units/ml IFN-γ or 100 units/ml mouse (mIL-9) or human IL-9). Cell viability was measured by FACS analysis after staining with propidium iodide (125 μg/ml). One significant experiment out of a total of four is shown. Error bars indicate the S.D. values measured from triplicate cultures. wt, wild-type.
glutamine 370; and STAT5 required leucine 368, in line with proposed consensus sequences (11, 17, 28, 29).

Based on these observations, we took advantage of the corresponding IL-9R mutants to assess the respective roles of STAT1, STAT3, and STAT5 in various IL-9 activities in vitro. The results are summarized in Table I. We first showed that induction of differentiation genes such as granzyme A, Ly-6A/E, and L-selectin could be mediated by STAT1 alone, although STAT3 (but not STAT5) may also be involved. Granzyme A has been identified as a gene induced by IL-9 (but not by IL-2) in the TS2 T cell clone (25). In these cells, IL-9 activates the same STAT proteins as in BW5147 cells, whereas IL-2 activates only STAT5.2 Thus, our data suggest that specific gene induction in this model could be due to a distinct STAT activation pattern. Moreover, IFN-γ and IL-6, cytokines that activated only STAT1 or both STAT1 and STAT3, also induced the expression of granzyme A and Ly-6A/E in BW5147 cells. Further analysis of the STAT-binding site in the Ly-6A/E promoter indicated that differences in DNA-binding properties of the different STAT proteins account for specific gene induction. Analysis of mice deficient in STAT1 and/or STAT3 should further establish the role of these STAT proteins in gene induction by IL-9.

While looking for proto-oncogenes regulated by IL-9, we observed that pim-1 kinase expression was up-regulated by IL-9. The role of this kinase in IL-9 signaling has to be further analyzed, particularly in hematopoietic cells, where pim-1 is predominantly expressed (36). IL-3 also induced pim-1 expression, as described by Mui et al. (34), who demonstrated a role for STAT5 in this process. Accordingly, pim-1 induction by IL-9 could be mediated by STAT5 alone, but also by STAT1 and/or STAT3. Noticeably, IFN-γ has been shown to regulate this gene via the binding of STAT1 on a GAS promoter element (26). Our data indicated that STAT3 and STAT5 are also able to bind to this GAS, which most likely participates in pim-1 regulation by IL-9 and IL-3. Interestingly, pim-1 expression is also induced by other cytokines activating STAT3 (IL-6) or STAT5 (IL-2, granulocyte/macrophage colony-stimulating factor). Thus, pim-1 expression in response to cytokines may be mediated by binding of STAT1, STAT3, or STAT5 to a single GAS promoter element, indicating redundancy between STAT proteins in this case.

We have recently shown that the activation of STAT factors is correlated with IL-9-induced proliferation of transfected Ba/F3 cells (11). The experiments presented here suggest that activation of a single STAT is sufficient to obtain a significant (but lower) IL-9 response, pointing to an additive effect of STAT factors. In line with our data, a potent constitutively active mutant of STAT5 has been shown recently to drive Ba/F3 proliferation in the presence of serum (38). Identification of the STAT-regulated genes that are responsible for proliferation should help to understand this effect.

Finally, analysis of protection against dexamethasone-induced apoptosis revealed functional redundancy between IL-9-activated STAT3 and STAT5. Moreover, the use of a constitutively activated STAT5 protein demonstrated that STAT activation alone is sufficient to protect against apoptosis. Interestingly, STAT5 is also activated by IL-2 and IL-7, which are other potent inhibitors of glucocorticoid-induced cell death (7). Recently, it was shown that IL-6 fails to prevent apoptosis in STAT3-deficient T cells (39), in agreement with our results. By contrast, STAT1 might not be involved in the process since IFN-γ did not inhibit apoptosis in our model.

We hypothesize that this effect of IL-9 is mediated by the induction of a gene via either STAT3 or STAT5. However, we failed so far to detect any change in the expression of well known inhibitors of apoptosis such as bcl-2, bcl-X, and iap family genes in BW5147 cells. Alternatively, STAT3 and STAT5 dimers have been shown to interact with the glucocorticoid receptor and, at least for STAT5, to repress glucocorticoid-mediated transcription (40, 41). However, IL-9 does not inhibit the expression of several corticoid-regulated genes in T cell clones (9). Further work will have to determine which mechanism may account for apoptosis inhibition by IL-9. In summary, the results reported here show that the activation of

---

**FIG. 10. A constitutively active variant of STAT5 inhibits apoptosis induced by dexamethasone.** BW5147 cells were stably transfected with a STAT5-VP16-JAK2 chimeric construct. Constitutive STAT5 activation was tested by EMSA with a GRR probe. Apoptosis in transfected with a STAT5-VP16-JAK2 chimeric construct. Constitutive STAT5 activation was tested by EMSA with a GRR probe. Apoptosis in

**TABLE I**

<table>
<thead>
<tr>
<th>Cytokine/receptor</th>
<th>Activated STAT</th>
<th>Granzyme A Ly-6A2</th>
<th>L-selectin</th>
<th>pim-1</th>
<th>Proliferation</th>
<th>Apoptosis inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-9/wild-type</td>
<td>1, 3, 5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-9/mut1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-9/mut6</td>
<td>1, 3</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-9/mut7</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-9/mut9</td>
<td>(1), 3, 5</td>
<td>+</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1</td>
<td></td>
<td>+</td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>IL-6</td>
<td>(1), 3</td>
<td></td>
<td>++&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-3</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> ND, not determined.
<sup>b</sup> IFN-γ and IL-6 have been reported to induce pim-1 in another system (26, 36).
<sup>c</sup> In murine T helper cell lines such as TS2 (8, 25).
distinct STAT factors in response to IL-9 plays both specific and redundant roles in the activity of this cytokine and that cooperation between STAT proteins may be required for some complex activities such as proliferation.

Acknowledgments—We thank Drs. R. Palacios, S. Nagata, A. Van Halteren, A. Burgess, and W. Fiers for generous donations of reagents.

REFERENCES

Distinct Roles for STAT1, STAT3, and STAT5 in Differentiation Gene Induction and Apoptosis Inhibition by Interleukin-9
Jean-Baptiste Demoulin, Emiel Van Roost, Monique Stevens, Bernd Groner and Jean-Christophe Renauld

doi: 10.1074/jbc.274.36.25855

Access the most updated version of this article at http://www.jbc.org/content/274/36/25855

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 22 of which can be accessed free at http://www.jbc.org/content/274/36/25855.full.html#ref-list-1