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Immunology

Restoration of surface IgM-mediated apoptosis in an anti-IgM-resistant variant of WEHI-231 lymphoma cells by HS1, a protein-tyrosine kinase substrate

(B cell/tyrosine phosphorylation/signal transduction/programed cell death)

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ABSTRACT **The HS1 protein is one of the major substrates of non-receptor-type protein-tyrosine kinases and is phosphorylated immediately after crosslinking of the surface IgM on B cells. The mouse B-lymphoma cell line WEHI-231 is known to undergo apoptosis upon crosslinking of surface IgM by anti-IgM antibodies. Variants of WEHI-231 that were resistant to anti-IgM-induced apoptosis expressed dramatically reduced levels of HS1 protein. Expression of the human HS1 protein from an expression vector introduced into one of the variant cell lines restored the sensitivity of the cells to apoptosis induced by surface IgM crosslinking. These results suggest that HS1 protein plays a crucial role in the B-cell antigen receptor-mediated signal transduction pathway that leads to apoptosis.**

Aggregational membrane-bound antigen receptors, such as surface immunoglobulin M (sIgM) on B cells, produce signals that may lead to proliferation, differentiation, apoptosis, or anergy of the cells (1). After crosslinking of the sIgM, one of the initial intracellular signaling events is the activation of non-receptor-type protein-tyrosine kinases (NR-PTKs), including $p53/56^{Lyn}$, $p55^{Blk}$, $p59^{Fyn}$, and $p72^{Syk}$ (2–4). These kinases rapidly phosphorylate tyrosine residues of a number of intracellular substrates, including phosphatidylinositol 3-kinase, phospholipases C-γ1 and -γ2, Ras GTPase-activating protein, and Vav (5–9). These phosphorylated substrates are thought to transmit further signals in cascades that eventually reach the nucleus, where the expression of immediate early genes, such as *c-myc*, *c-fos*, or *egr-1*, is induced (10–12). NR-PTKs and their substrates often contain Src homology 2 (SH2) and/or Src homology 3 (SH3) domains, which mediate intra- or intermolecular association by binding to their specific target motifs (13–15).

HS1 is a 75-kDa protein found in the cytoplasm and to a lesser extent in the nucleus of essentially all hematopoietic cells (16). The amino acid sequence of HS1 is highly conserved between human and mouse and contains an SH3 domain, which has recently been shown to bind to a specific proline-rich sequence, at its carboxyl terminus. In its amino-terminal half, HS1 contains a three and one-half times repeat of a unique 37-amino acid-residue motif; each motif contains a helix–turn–helix structure similar to a prokaryotic DNA-binding motif (16, 17). HS1 also possesses features characteristic of a transcription factor—e.g., a putative nuclear localizing signal, acidic and amphipathic α-helix structure (18), and a basic motif resembling the DNA-binding domain of Jun, Fos, Myc, and other helix–loop–helix protein families (19). Recently, we showed that the HS1 protein was associated with the Src-like protein-tyrosine kinase Lyn and was tyrosine-phosphorylated shortly after crosslinking of the sIgM on a human B-cell line (20). The tyrosine-phosphorylated HS1 was shown to be physically associated with SH2 domains of Src-like protein-tyrosine kinases, such as Lyn, Blk, or Fyn, with the highest affinity among the tyrosine-phosphorylated proteins in sIgM-crosslinked B cells (20, 21). These data suggest that the HS1 protein in B cells may be involved as a substrate of NR-PTKs in a signal transduction pathway from sIgM.

B-cell tolerance is achieved by elimination (clonal deletion) or inactivation (clonal anergy) of self-reactive cells (22, 23). Clonal deletion is thought to be mediated by apoptosis of newly formed B cells that have received a signal through sIgM (24–27). The signal transduction mechanism leading to this apoptosis is largely unknown, but it has recently been shown that Blk, one of the NR-PTKs associated with sIgM, may be involved in the apoptotic signal, since an antisense Blk oligonucleotide inhibited apoptosis of a B-lymphoma induced by anti-IgM antibody treatment (29). Although a number of intracellular substrates of NR-PTKs have been identified, none of them has so far proved to be functionally involved in the induction of lymphocyte responses such as apoptosis induced by sIgM crosslinking.

Crosslinking of sIgM on WEHI-231 B-lymphoma cells induces growth inhibition and apoptosis as evidenced by nuclear fragmentation and oligosomal DNA cleavage. Thus, WEHI-231 cells have been often used as a model system to study the mechanism of the antigen receptor-induced apoptosis (30, 31). In the present study, we analyzed two variants of the WEHI-231 cell line (M1 and M22) that were selected for growth in the presence of anti-IgM antibodies (32) and demonstrated that the HS1 protein may be one of the components involved in the sIgM-mediated apoptosis of WEHI-231 cells. To our knowledge, there have been no previous reports that a substrate of NR-PTKs associated with surface antigen receptors functionally mediates apoptosis of the B cells by transmitting the signal from the receptors.

MATERIALS AND METHODS

Cells. Generation of the anti-IgM antibody-resistant variants of WEHI-231 cells has been described (32). WEHI-231 cells and the variants were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 50 μM 2-mercaptoethanol (2-ME), and antibiotics. ΨCRE cells and NIH 3T3 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf serum, 50 μM 2-ME, and antibiotics.

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Abbreviations: sIgM, surface IgM; NR-PTKs, non-receptor-type protein-tyrosine kinases; SH3, Src homology 3; hHS1, human HS1.

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Fluorescence-Activated Cell Sorting Analysis for Apoptosis. Cells ($1–2 \times 10^5$) were cultured with (+) or without (−) 8 μg of affinity-purified goat antibody to mouse IgM per ml (Cappel) in 1–2 ml of culture medium for 48 hr, fixed with 70% ethanol at 4°C for 1 hr, and then incubated with phosphate-buffered saline (PBS) containing 50 μg of RNase A per ml (Sigma) at 37°C for 20 min. DNA content of the cells was measured after staining with 50 μg of propidium iodide per ml by flow cytometry using the FACScan (Becton Dickinson) as described (33). Cells containing less DNA than those in G_0/G_1 phase represent apoptotic cells.

Retroviral Infection. The retroviral vector pM5-hHS1 was constructed by cloning the wild-type human HS1 (hHS1) cDNA *Eco*RI fragment into the *Eco*RI-digested pM5-neo plasmid (34). The ecotropic packaging cell line ΨCRE (a generous gift from R. C. Mulligan, Massachusetts Institute of Technology, Cambridge) (35) was transfected with pM5-hHS1 or pM5-neo for mock transfection by the calcium phosphate coprecipitation method. To obtain clones that produce high titers of retroviruses, we used the reinfection procedure as follows. ΨCRE cells, pretreated with 800 ng of tunicamycin per ml (Sigma) for 12 hr, were infected with the transient supernatants of pM5-hHS1 or pM5-neo transfected ΨCRE cells in the presence of 8 μg of Polybrene per ml (Sigma) and were then selected in DMEM supplemented with 10% calf serum and 1 mg of G418 per ml (Sigma). Among the drug-resistant clones, high retrovirus-producing ΨCRE clones were selected by their efficiency for infecting NIH 3T3 cells. The titer of the clones selected was $>1 \times 10^6$ colony-forming units/ml. Infection of the WEHI-231 variants was performed by coculture with irradiated (2000 rads; 1 rad = 0.01 Gy) ΨCRE clones in the presence of 4 μg of Polybrene per ml for 2–4 days. Stable infectants of the variants were selected in culture medium containing 800 μg of G418 per ml. The drug-resistant clones were analyzed by Western blot assay for the expression of hHS1 protein, and the clones with the highest expression of hHS1 protein were used in the present study.

Western Blot Analysis. Cells (1×10^7) in 1 ml of serum-free RPMI-1640 medium were incubated with or without 20 μg of affinity-purified goat antibody to mouse IgM at 37°C for 1 min. The cells were then lysed in SDS/PAGE sample buffer containing 2-ME and 2 mM sodium orthovanadate, and the lysates were subjected to Western blot analysis with an anti-phosphotyrosine monoclonal antibody, PY20, labeled with horseradish peroxidase (HRP) (Transduction Laboratories, Lexington, KY), with a rat antiserum specific for mouse HS1 (against the peptide sequence $Ala^{306}–Arg^{320}$, unpublished) followed by HRP-labeled rabbit anti-rat IgG (Zymed) or with a rabbit antiserum specific for hHS1 (against the peptide sequence $Val^{306}–Ser^{320}$; ref. 16) followed by HRP-labeled goat anti-rabbit IgG (Zymed). The blots were developed by the ECL system (Amersham). Molecular mass standards (97 kDa, 68 kDa, and 50 kDa) were purchased from GIBCO.

DNA Fragmentation Assay. The DNA fragmentation assay was performed as described with slight modifications (36). Cells ($1–2 \times 10^6$) were incubated with or without 6 μg of affinity-purified goat antibody to mouse IgM per ml for 48 hr. The cells were washed once with PBS and then lysed in 500 μl of a hypotonic buffer containing 10 mM Tris·HCl, 10 mM EDTA, and 0.2% Triton X-100 (pH 7.5). The lysates were centrifuged at $10,000 \times g$ for 10 min at 4°C. The supernatants containing low molecular mass DNA were treated with 200 μg of RNase A per ml at 37°C for 1 hr and with 400 μg of proteinase K per ml at 55°C for 2 hr and then extracted with phenol/chloroform/isoamyl alcohol (24:1). Pellets containing high molecular mass DNA were lysed in 500 μl of a buffer containing 50 mM Tris·HCl, 100 mM NaCl, 100 mM EDTA, and 1% SDS (pH 8.0) and then treated with RNase A and proteinase K as above. Nucleic acids were precipitated with ethanol and dissolved in 10 mM Tris·HCl/1 mM EDTA/1% SDS, pH 7.5. After incubation at 60°C for 10 min, the samples (1×10^6 cell equivalents per lane) were electrophoresed at 60 V for 6 hr on a 1.5% agarose gel in TBE buffer. DNA was visualized by ethidium bromide staining. *Hae* III-digested ϕX174 (300 ng) was used for molecular mass markers.

Cell Proliferation Assay. Cell proliferation was estimated by [^{3}H]thymidine incorporation assay as described (37). Briefly, cells (2×10^4) were cultured in triplicate with or without 10 μg of affinity-purified goat antibody to mouse IgM per ml in 200 μl of culture medium for 48 hr. The cultures were pulsed with 0.5 μCi of [^{3}H]thymidine (DuPont; 1 Ci = 37 GBq) for the last 4 hr of the culture.

RESULTS

Variants of WEHI-231 Cells, M1 and M22, Are Resistant to sIgM-Induced Apoptosis. Recently, two variant lines (M1 and M22), which are resistant to apoptosis induced by sIgM crosslinking, have been generated from the WEHI-231 cell line. In contrast to the parental WEHI-231 cells, anti-IgM antibody treatment only moderately affected the proliferation of M1 cells and had no effect on that of M22 cells (38) and did not induce apoptosis in either M1 or M22 cells as assayed by DNA fragmentation (32). The resistance of these cells to sIgM-induced apoptosis was confirmed in the following assay. The wild-type and the variant cells were incubated with polyclonal anti-IgM antibodies for 48 hr, fixed with ethanol, and then incubated for 20 min to allow release of small fragmented DNA from the cells. After staining with propidium iodide, the cells were analyzed for DNA content by flow cytometry as described (33). Apoptotic cells contain less DNA than the cells in G_0/G_1 phase due to the DNA fragmentation. As shown in Fig. 1, after treatment of parental WEHI-231 cells with anti-IgM antibodies, the proportion of the apoptotic cells greatly increased (from 12% to 76%). By contrast, the same treatment induced only a marginal increase in the apoptotic cell population of M1 cells (from 8% to 16%) and essentially no increase in M22 cells (from 6% to 8%).

Expression of the hHS1 Protein Restores the Sensitivity of M1 Cells to Apoptosis. The HS1 protein is associated with NR-PTKs, such as Lyn, and is tyrosine-phosphorylated shortly after crosslinking of sIgM (20, 21). This indicates that, in B cells, the HS1 protein may be involved in a signal transduction pathway from the sIgM as a substrate of protein tyrosine kinase(s). As we reported previously (38), the M1 and M22 anti-IgM-resistant variants expressed an extremely low level of the HS1 protein, >50-fold less than the parental WEHI-231 cells (Fig. 2*A Lower*). Although tyrosine phosphorylation of the HS1 protein was greatly reduced in the variant cells (Fig. 2*A Upper*), the reduction

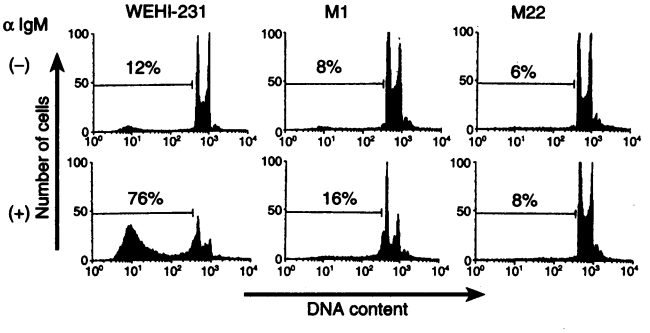

FIG. 1. The M1 and M22 variants of WEHI-231 are resistant to apoptosis induced by crosslinking of sIgM. Cells were incubated with (+) or without (−) affinity-purified goat antibody to mouse IgM (α IgM) for 48 hr, treated and stained with propidium iodide, and analyzed by flow cytometry. The DNA content of the non-gated population was expressed as the histogram of propidium iodide fluorescence intensity. The percentage of apoptotic cells (defined by horizontal bars) is denoted in each histogram.

is due to the limited amounts of HS1 protein present in these variant cells rather than to inefficient tyrosine phosphorylation, since the degree of phosphorylation per molecule in the variants was essentially the same as in the wild-type cells (data not shown). Reduction of the tyrosine phosphorylation and/or expression level of some other proteins in M1 and M22 cells is more evident in the present analysis with a particular anti-phosphotyrosine antibody (PY20) than in the analysis with the other antibody (4G10) reported previously (38).

To determine whether the reduction of HS1 expression contributed to the resistance of these variant cells to apoptosis, we introduced the hHS1 cDNA into the variants by retroviral infection (34, 35). Independent drug-resistant clones, with independent virus-integration events, were isolated from the infected M1 and M22 variants. Western blotting with antibodies against hHS1 showed that the stable infectants expressed the hHS1 protein (representative data are shown in Fig. 2*B Lower*). The hHS1 expression level was comparable to that of the human B-lymphoma cell line Daudi (data not shown). The hHS1 protein was tyrosine-phosphorylated 1 min after crosslinking of sIgM in these cells (Fig. 2*B Upper*). The reduced tyrosine phosphorylation (or expression) of other proteins was not restored in these infectants, suggesting that the reduction of tyrosine phosphorylation of such proteins was not due to the deficiency of HS1. To assess their sensitivity to apoptosis, the hHS1-introduced variant cells were incubated with the same anti-IgM antibodies as above for 48 hr and the DNA content in the cells was analyzed by flow cytometry. As shown in Fig. 3*A*, the apoptotic cell population markedly increased in the two independent hHS1-introduced M1 cell clones (59% and 52%) after anti-IgM treatment, in contrast to the mock-infected M1 cells (15%). Analysis by gel electrophoresis showed that DNA fragmentation occurred in hHS1-introduced M1 cells after crosslinking of sIgM (Fig. 3*B*). On the other hand, hHS1-introduced M22 cells remained resistant to sIgM-mediated apoptosis (Fig. 3*A*). The restoration of sensitivity to anti-IgM-induced apoptosis by the expression of hHS1 in M1 cells was also confirmed by a [^{3}H]thymidine incorporation assay. As shown in Fig. 3*C*, DNA synthesis of M1 cells expressing the hHS1 was reduced following anti-IgM treatment, while that of M22 cells remained unaffected in the presence of hHS1.

These results indicate that the reduced expression of HS1 protein is the major cause for the resistance of the M1 cells to the sIgM-mediated apoptosis, but that of M22 cells seems to include additional defects that cannot be compensated for by expression of hHS1. Thus, it is concluded that HS1 is necessary for the induction of apoptosis in WEHI-231 cells by the sIgM crosslinking, although not sufficient for it.

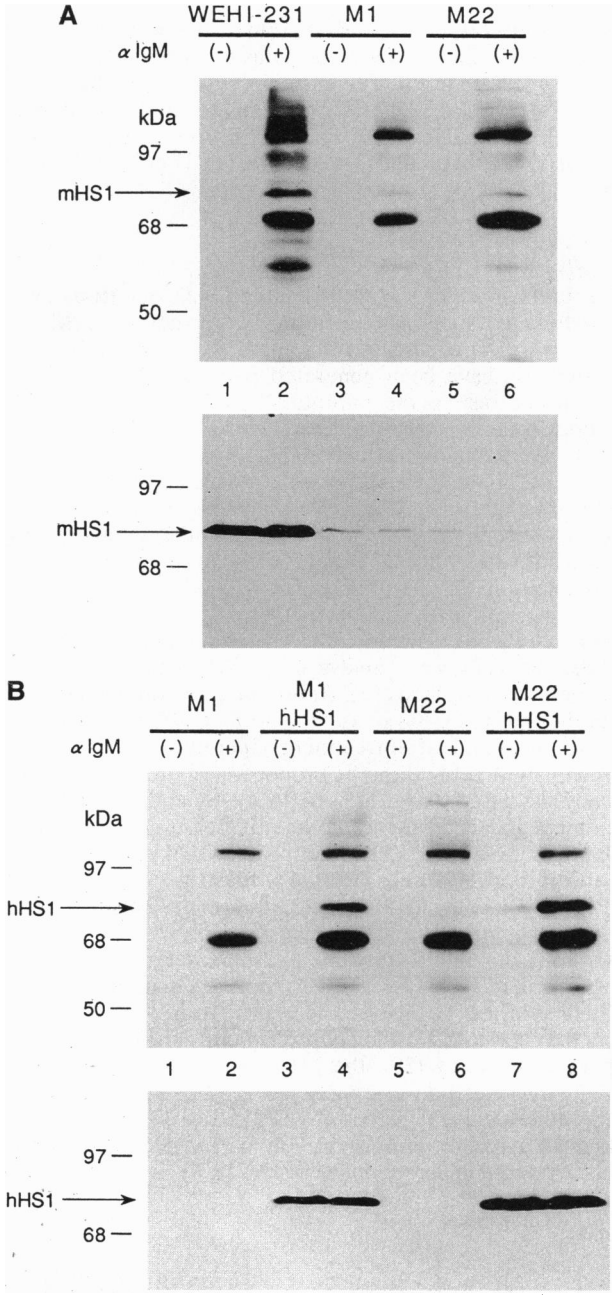

FIG. 2. Exogenous hHS1 protein is tyrosine-phosphorylated shortly after sIgM crosslinking in M1 and M22 cells. (*A*) WEHI-231 cells and the variants were incubated with (lanes 2, 4, and 6) or without (lanes 1, 3, and 5) affinity-purified goat antibody to mouse IgM for 1 min, lysed with SDS/PAGE sample buffer, and analyzed by Western blot using an anti-phosphotyrosine monoclonal antibody (*Upper*) or a rat antiserum specific for mouse HS1 (mHS1) (*Lower*). The position of the mouse HS1 protein band is indicated by an arrow. Molecular masses of standard proteins are indicated. (*B*) Uninfected M1 and M22 cells and hHS1 infectants were incubated with (lanes 2, 4, 6, and 8) or without (lanes 1, 3, 5, and 7) affinity-purified goat antibody to mouse IgM for 1 min and the cell lysates were subjected to Western blot analysis using the anti-phosphotyrosine monoclonal antibody (*Upper*) or a rabbit antiserum specific for hHS1 (*Lower*). Lanes 3 and 4, hHS1-introduced M1 cells (M1-H11); lanes 7 and 8, hHS1-introduced M1 cells (M1-H11); lanes 7 and 8, hHS1-introduced M22 cells (M22-H12). The position of the hHS1 protein band is indicated by an arrow. Molecular masses of standard proteins are indicated.

DISCUSSION

The importance of NR-PTKs for antigen receptor-mediated signals was suggested by the facts that tyrosine kinase inhibitors, such as herbimycin A or genistein, blocked activation of phospholipase C and the Ras pathway and inhibited proliferation of lymphocytes (39–41). Genistein was also shown to inhibit activation-induced cell death of a T-cell hybridoma (42). We were unable to evaluate the effects of these tyrosine kinase inhibitors on the anti-IgM-induced apoptosis due to their toxicity for WEHI-231 cells (unpublished data). However, it has been reported that one of NR-PTKs associated with sIgM—namely, Blk—may mediate the signal leading to apoptosis, since an antisense oligonucleotide corresponding to the Blk mRNA inhibited apoptosis induced by anti-IgM antibody treatment of a B lymphoma (29). Interestingly, an antisense oligonucleotide corresponding to the Lyn mRNA inhibited anti-IgM-induced cell cycle arrest, but not apoptosis, of a B lymphoma (43). These data indicate that NR-PTKs play an important role in signal transduction leading to growth arrest or apoptosis.

Here we demonstrated that HS1, a substrate of NR-PTK(s) coupled to sIgM on B cells, was necessary, if not sufficient, for the induction of apoptosis by the sIgM crosslinking of WEHI-231 cells. We utilized two anti-IgM antibody-resistant variants

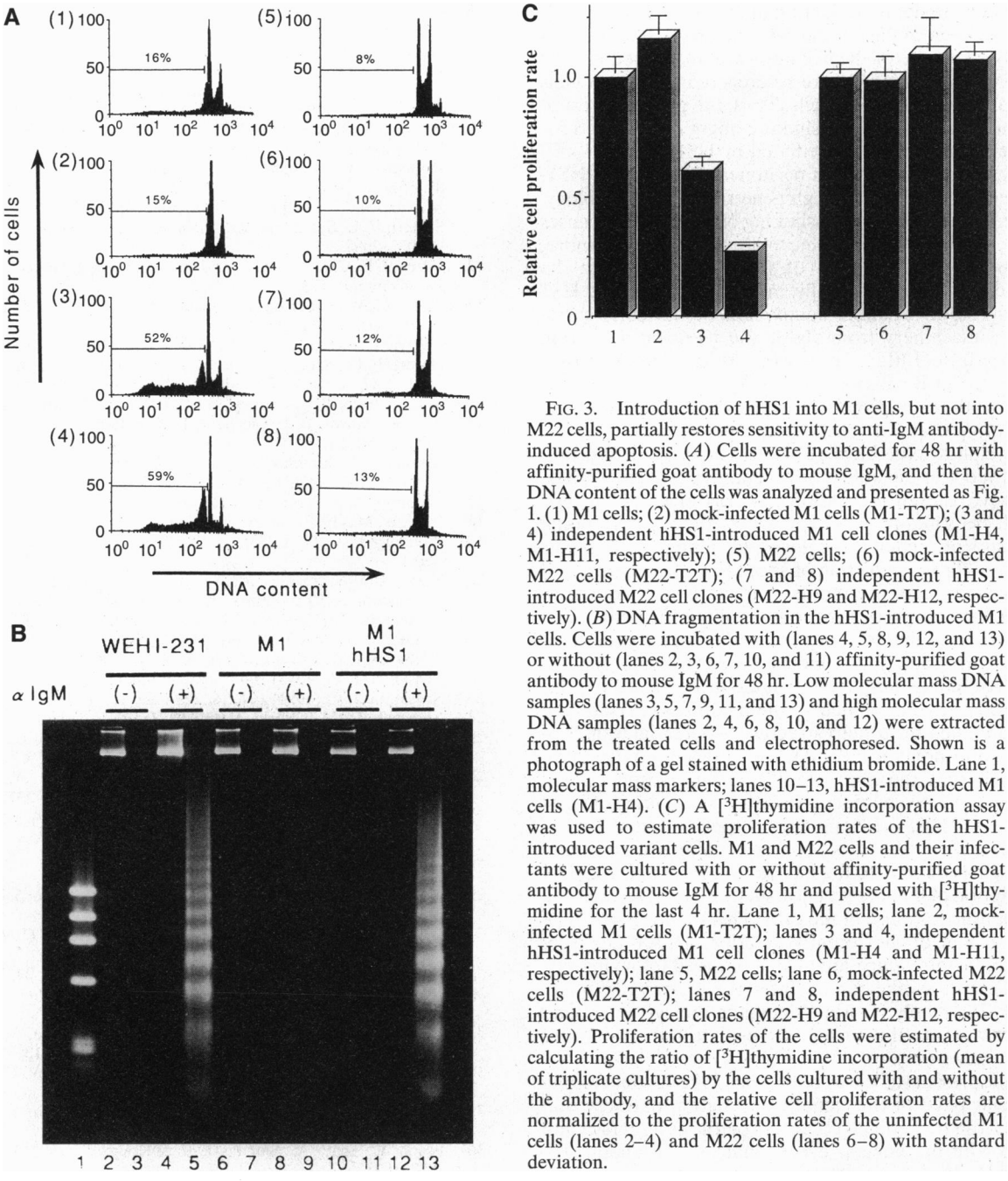

FIG. 3. Introduction of hHS1 into M1 cells, but not into M22 cells, partially restores sensitivity to anti-IgM antibody-induced apoptosis. (*A*) Cells were incubated for 48 hr with affinity-purified goat antibody to mouse IgM, and then the DNA content of the cells was analyzed and presented as Fig. 1. (1) M1 cells; (2) mock-infected M1 cells (M1-T2T); (3 and 4) independent hHS1-introduced M1 cell clones (M1-H4, M1-H11, respectively); (5) M22 cells; (6) mock-infected M22 cells (M22-T2T); (7 and 8) independent hHS1-introduced M22 cell clones (M22-H9 and M22-H12, respectively). (*B*) DNA fragmentation in the hHS1-introduced M1 cells. Cells were incubated with (lanes 4, 5, 8, 9, 12, and 13) or without (lanes 2, 3, 6, 7, 10, and 11) affinity-purified goat antibody to mouse IgM for 48 hr. Low molecular mass DNA samples (lanes 3, 5, 7, 9, 11, and 13) and high molecular mass DNA samples (lanes 2, 4, 6, 8, 10, and 12) were extracted from the treated cells and electrophoresed. Shown is a photograph of a gel stained with ethidium bromide. Lane 1, molecular mass markers; lanes 10–13, hHS1-introduced M1 cells (M1-H4). (*C*) A [^{3}H]thymidine incorporation assay was used to estimate proliferation rates of the hHS1-introduced variant cells. M1 and M22 cells and their infectants were cultured with or without affinity-purified goat antibody to mouse IgM for 48 hr and pulsed with [^{3}H]thymidine for the last 4 hr. Lane 1, M1 cells; lane 2, mock-infected M1 cells (M1-T2T); lanes 3 and 4, independent hHS1-introduced M1 cell clones (M1-H4 and M1-H11, respectively); lane 5, M22 cells; lane 6, mock-infected M22 cells (M22-T2T); lanes 7 and 8, independent hHS1-introduced M22 cell clones (M22-H9 and M22-H12, respectively). Proliferation rates of the cells were estimated by calculating the ratio of [^{3}H]thymidine incorporation (mean of triplicate cultures) by the cells cultured with and without the antibody, and the relative cell proliferation rates are normalized to the proliferation rates of the uninfected M1 cells (lanes 2–4) and M22 cells (lanes 6–8) with standard deviation.

of WEHI-231 cells, M1 and M22, that expressed a dramatically reduced level of HS1 protein. The results indicated that introduction of HS1 restored the sensitivity of M1 cells, but not of M22 cells, to the anti-IgM antibody treatment. The resistance of M1 and M22 cells to sIgM-mediated apoptosis does not seem to stem from a general defect in the induction of apoptosis since exposure of both variants as well as the parental WEHI-231 cells to the Ca^{2+} ionophore A23187 could induce apoptosis (32). Bcl-2, a protein known to block a broad range of apoptotic pathway, also may not be involved since the variant cells express Bcl-2 at the same level as the parental WEHI-231 cells before and after the crosslinking of sIgM (32). Moreover, apoptosis of WEHI-231 cells induced by sIgM crosslinking was not inhibited by overexpression of the introduced *bcl-2* gene (44).

Although the levels of IgM expression on the surface are similar in all three cell lines, the kinetics of tyrosine phosphorylation of some other proteins, in addition to HS1, differed from each other and from the parental cells after sIgM crosslinking (Fig. 2*A* and ref. 38). Also, the increase of intracellular Ca^{2+} concentration induced by sIgM crosslinking was reduced in the variants compared to the parental cells, suggesting a partial defect in Ca^{2+} signaling (38). Thus, other signaling molecules in addition to HS1 could be differentially deficient in these variants. Despite this, introduction of mere HS1 restored the sIgM-mediated apoptosis of M1 cells, indicating a pivotal role of HS1 in the pathway leading to the apoptosis of WEHI-231 cells among such molecules. However, the incomplete restoration of the apoptosis in M1 cells by the introduction of HS1 may indicate that the other molecule(s) functions synergistically with HS1 in this pathway. The failure to reverse the resistance in M22 cells by introduction of HS1 suggests that M22 cells, but not M1 cells, are deficient also in molecule(s) that is prerequisite for apoptosis of the cells. In this regard, it is noteworthy that M22 cells barely suffered from growth arrest by sIgM crosslinking, in contrast to M1 cells,

which showed a moderate reduction of proliferation rate (38). This is also evident in Figs. 1 and 3*A*. The proportions of cells in S and G_2/M phases of the cell cycle are unaffected in M22 cells by sIgM crosslinking but are severely reduced in M1 cells as in the parental WEHI-231 cells. Thus, the growth arrest of the cells induced by sIgM crosslinking might be required for subsequent apoptosis that is mediated by HS1.

Recently, we have found that peritoneal B cells from HS1-deficient mice, generated through gene-targeting technology, are resistant to multivalent crosslinking of sIgM, which causes apoptosis of those cells in the normal mouse. Furthermore, anti-IgM-induced proliferation of splenic B cells from the HS1-deficient mice was partially impaired (28). Thus, HS1 protein may be an essential component of the early signal transduction machinery from sIgM, and its down-regulation would impair cellular responses that follow crosslinking of sIgM in B cells.

De novo synthesis of protein is required for sIgM-mediated apoptosis of WEHI-231 cells, since the protein synthesis inhibitor cycloheximide blocked this response (36). We previously showed that the anti-IgM-resistant variants of WEHI-231 cells have altered expression patterns of c-*myc* mRNA after sIgM crosslinking (32). Therefore, the transcriptional activation and/or regulation of genes, including c-*myc*, seems to be required for the induction of apoptosis. The HS1 protein possesses several sequence motifs characteristic of transcription factors, including repeating motifs containing helix–turn–helix structures similar to those in several prokaryotic transcription factors (16, 17), a sequence similar to the consensus for the DNA-binding motifs found in the proteins of Jun, Fos, Myc, and other helix–loop–helix protein families (19), and the acidic and amphipathic α-helix structure, a potential transcriptional activation domain (18). Also, our recent studies have demonstrated that the amount of tyrosine-phosphorylated HS1 protein in the nucleus increases after crosslinking of sIgM (unpublished). Thus, it is tempting to speculate that HS1 is phosphorylated by NR-PTKs upon crosslinking of sIgM and transmits signals directly to the nucleus, where it may regulate the transcription of the target genes, such as c-*myc*, responsible for the apoptotic response of the cells. This situation would be analogous to that of the recently described STAT family of transcription factors, which are phosphorylated following various stimuli, such as interferons, interleukins, or growth factors, by Jak-Tyk family tyrosine kinases in the cytoplasm and then translocate into the nucleus to regulate transcription of their target genes (45).

Finally, the present study may offer a clue to better understand the molecular basis of the signal transduction pathway that is initiated from the antigen receptors on the cell surface and terminated with the nuclear events that lead to apoptosis.

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