Interferon-α–Inducible Proteins Are Novel Autoantigens in Murine Lupus

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Objective. To investigate the spectrum of B cell autoimmunity in the recently described anti-CD1–autoreactive T cell receptor (TCR)–transgenic murine lupus-like (CD1 lupus-like) model.

Methods. Lethally irradiated BALB/c/nu/nu mice were injected intravenously with donor BALB/c bone marrow and spleen cells expressing TCRα/H9251 and TCRβ transgenes that recognize CD1d. Sera from adoptive host animals that developed lupus (i.e., CD1 lupus mice) were collected at serial time points and analyzed by Western blotting and immunoprecipitation, using protein extracts prepared from NIH3T3 mouse fibroblasts and EL-4 lymphocytes, respectively. Sera obtained from older animals in several models of spontaneous lupus (NZB/NZW, MRL/H11545/H11545, and MRL/lpr mice), unmanipulated BALB/c/nu/nu mice, and normal BALB/c mice were used as controls.

Results. Analyses demonstrated that the prominent targets of autoantibodies in the CD1 lupus-like model are interferon-α (IFNα)–inducible antigens. Biochemical and serologic characterizations identified one antigen as belonging to the interferon-inducible 202 (Ifi202) subfamily of proteins within the Ifi200 family, and a second antigen as a member of the 70-kd heat-shock protein family. Autoantibodies directed against these antigens were rapidly produced at an early stage of disease. Anti-p50 autoantibodies were present in sera from 7 (78%) of 9 CD1 lupus mice that developed severe kidney disease.

Conclusion. IFNα-inducible proteins represent a novel class of autoantigens in murine lupus, and the findings suggest additional roles for IFNα in this disease. Since Ifi202 autoantigens are encoded by the murine non–major histocompatibility complex lupus-susceptibility gene locus Ifi202, these data provide a link between recent advances in lupus genetics and the formation of autoantibodies.

Although a wide spectrum of autoantibodies has been identified in systemic lupus erythematosus (SLE), only a few, such as anti-DNA antibodies, have been implicated in the pathogenesis of certain manifestations of this complex disease (1). Other unidentified autoantibodies may play potentially crucial roles with regard to tissue injury, and their identification may provide critical insights into the pathogenesis of connective tissue disease (2).

CD1 is a non–major histocompatibility complex (MHC)–encoded antigen-presenting molecule that is primarily involved in the presentation of glycolipid antigens to subsets of T cells expressing natural killer cell markers (natural killer T cells) (3). Some reports have suggested that CD1 presentation of nonlipid antigens is possible (4,5). The physiologic ligands of CD1 are still unknown. Evidence is accumulating to support the notion that the CD1 molecule is important in the modulation of immune responses in several autoimmune diseases (6). We recently suggested a role for CD1 in the pathogenesis of hereditary murine lupus in NZB/NZW mice (7). In the anti-CD1 T cell receptor (TCR)–transgenic murine lupus-like model (referred to as the CD1 lupus-like model), the transfer of a single-positive
(SP) (CD4+ or CD8+) subset of transgenic T cells into BALB/c/nu/nu animals activates syngeneic host B cells to produce anti–double-stranded DNA (anti-dsDNA) autoantibodies (8) and induces the rapid onset of glomerulonephritis (GN). The SP transgenic T cells express a Th1-like cytokine pattern. Double-negative (DN) (CD4−,CD8−) transgenic T cells, which express a Th2-like pattern, do not induce lupus GN (8).

To further elucidate the humoral autoimmune response in the CD1 lupus-like model, we investigated the possibility that autoantibodies other than anti-dsDNA antibodies are produced. We used immunoprecipitation (IP) and Western blot (WB) analyses to screen sera of CD1 lupus mice for their ability to recognize novel autoantigens. We identified novel, interferon-inducible factors that comigrate, as detected on WB, with the gene products recently identified in the 70-kd heat-shock protein (Hsp70) family as well as 2 novel autoantigens. We identified novel, interferon-inducible proteins that may represent a new class of autoantigen targets in this disease.

MATERIALS AND METHODS

Transgenic mice. Development of SP and DN lines of TCR α- and β-chain gene–transgenic mice has been described in detail previously (16). Briefly, 2 lines of transgenic mice were established using the TCR α-chain (Vα4.4-Jα24) and β-chain (Vβ9-Dβ1.1-Jβ2.1) genes from a cloned CD4+–CD8− (DN) T cell line derived from the spleen of a BALB/c mouse (16). The TCR genes were expressed in CD4+,CD8− and CD4−,CD8+ (SP) T cells and double-positive (DP) T cells in the thymus and in SP T cells in the peripheral lymphoid tissues and bone marrow in one transgenic mouse line, and predominantly in DN T cells in the other line (16). In previous in vitro experiments, we demonstrated that this particular clone of transgenic CD4+ and CD8+ cells proliferated in response to syngeneic B cells expressing CD1, but did not proliferate in response to nontransfected control B cells lacking CD1 expression (8). These experiments were performed using a BALB/c B cell line (A20) that had been transfected with complementary DNA encoding CD1 (a gift from M. Kronenberg, La Jolla Institute for Allergy and Immunology, La Jolla, CA). In the experiments reported herein, we used CD1 TCR–transgenic mice that were backcrossed to BALB/c mice for at least 10 generations; 2–3-month-old male mice were used as cell donors.

Induction of autoimmune disease. Lethally irradiated (800 cGy whole-body irradiation), 2–3-month-old male host BALB/c/nu/nu mice received 5 × 10^6 SP transgenic donor bone marrow cells intravenously, along with 2 × 10^5 sorted, SP transgenic donor spleen CD4− or CD8+ T cells within 24 hours after irradiation. Briefly, the bone marrow and spleen cells were stained with saturation concentrations of phycoerythrin (PE)–conjugated anti-CD4 (GK1.5) and PE-conjugated anti-CD8 (anti-Lyt2) monoclonal antibodies (obtained from Caltag, Burlingame, CA), since the TCRβ transgene uses the Vβ9 gene segment (8). Counterstaining was performed using fluorescein isothiocyanate–conjugated anti-TCRα/β (H57-597) or anti-Vβ9 (MR10-2) monoclonal antibodies, obtained from PharMingen (San Diego, CA). Previous studies showed that the SP transgenic T cells contained within the bone marrow cell inoculum of the experimental animals, and that the addition of sorted splenic T cells increases the rapidity and severity of disease activity (8).

For the serologic studies reported herein, 2 groups of mice were injected in separate experiments. In group A, of the 6 adoptive host mice that were injected with SP transgenic bone marrow and splenic T cells, 4 developed lupus with GN. In group B, of the 8 adoptive host mice that were injected with SP transgenic bone marrow and splenic T cells, 5 developed lupus with GN.

Serum. Serum from the adoptive hosts of group A was obtained serially at days 0, 20, 35, 45, 60, and 75 after the adoptive transfer procedure. Serum from the adoptive hosts of group B was obtained at days 0, 45, and 60. These sera and additional sera from unmanipulated wild-type BALB/c/nu/nu adoptive hosts that received DN transgenic cells, as well as sera from NZB/NZW, MRL+++, MRL/lpr, and normal BALB/c mice were stored at −80°C until used. Proteinuria was measured as described previously (8). Proteinuria of more than 100 mg/dl, measured in 2 separate determinations, was considered to be the cutoff level for positivity.

Antibodies. The mouse polyclonal antibodies were a gift from Dr. W. J. van Venrooij, University of Nijmegen, Nijmegen, The Netherlands (anti-U1A/U2B′94A9), from Drs. D. Choubey and P. Lengyl, Yale University, New Haven, CT (rabbit anti-mouse Ifi202 [anti-Ifi202(PL)]), and from Drs. G. Gribaudo and S. Landolfi, University of Turin, Turin, Italy (rabbit anti-mouse Ifi203). Anti-Hsp70/Hsc70 mouse monoclonal antibodies were purchased from StressGen, San Diego, CA. Goat anti-Ifi202 polyclonal antibody (M-15) was purchased from Santa Cruz Biotechnology (anti-Ifi202[SC]; Santa Cruz, CA).

Cell culture. NIH3T3 cells and EL-4 cells were grown in 5% CO2 at 37°C, using RPMI 1640 (for EL-4 cells) and Dulbecco’s modified Eagle’s medium (for 3T3 cells) (Gibco BRL, Grand Island, NY). Medium was supplemented with 9% heat-inactivated fetal bovine serum (for EL-4 cells) or bovine calf serum (for 3T3 cells) (BioWhittaker, Walkersville, MD), penicillin, and streptomycin (Gibco BRL). Cells were grown and harvested at mid-log phase (for EL-4) or 80% confluency (for 3T3), unless indicated otherwise.

Metabolic labeling. The 3T3 cells were incubated at 80% confluency, and the EL-4 cells were incubated at a density
of 1 × 10^6 cells/ml in labeling medium containing the following: 90% RPMI 1640 lacking either phosphate or methionine and cysteine (Gibco BRL), and 10% heat-inactivated fetal calf serum that had been dialyzed to equilibrium against a buffer containing 10 mM HEPES and 140 mM NaCl (Sigma, St. Louis, MO). Either [32P]orthophosphate or [35S]-methionine and cysteine (NEN, Boston, MA) were added at a concentration of 0.15 mCi/ml and 0.25 mCi/ml, respectively. Cells were then incubated at 37°C for 5 hours.

Cell lysis and activation with IFNα. Lysis of cells was performed as described previously (17). Recombinant murine IFNα (Calbiochem, La Jolla, CA) was used for all antigen-induction experiments at a concentration of 750 IU/ml culture medium. NIH3T3 and EL-4 cells were treated with IFNα for 18 hours, beginning at 50% confluence in the case of 3T3 cells or at 8 × 10^5 cells/ml in the case of EL-4 cells, followed by 5 hours of supplementation of the medium with fresh IFNα at 750 units/ml during metabolic labeling.

IP and WB analyses. Lysates were precleared 3 times for 15 minutes with 15 μl of a 50% solution of protein A-Sepharose (Pharmacia, Uppsala, Sweden) in phosphate buffered saline (PBS) and 2.5 μg rabbit anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA). IP was performed in 1% Nonidet P40 (NP40) lysis buffer followed by rotation in a cold room overnight. Precipitates were harvested by centrifugation for 15 seconds at 13,000 revolutions per minute and washed 3 times with NP40 lysis buffer, resuspended in sodium dodecyl sulfate (SDS) loading buffer with 4% 2-mercaptoethanol, boiled for 2 minutes, and electrophoresed on 12% or 7% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gels.

Proteins were transferred to nitrocellulose (Schleicher & Schuell, Keene, NH) for WB experiments or to polyvinylidene difluoride (PVDF; DuPont-New England Nuclear, Boston, MA) for phospho–amino acid analysis, and either exposed for autoradiography or subjected to WB analysis. For the IP and WB studies, nitrocellulose membranes were excised and blocked in 3% fat-free dry milk in PBS at room temperature for 1 hour, or at 4°C overnight. Anti-Hsp70/Hsc70 monoclonal antibodies (StressGen) were used at a dilution of 1:5,000 in 3% fat-free dry milk in PBS. Bands were detected using donkey anti-mouse IgG, conjugated to horseradish peroxidase (HRP; Jackson ImmunoResearch), and visualized using enhanced bioluminescence (Pierce, Rockford, IL), in accordance with the manufacturer’s specifications. For WB detection of autoantibodies against p70 and Hsp70/Hsc70, strips of nitrocellulose membrane were blocked as before and incubated with 1:200 dilutions of the same autoimmune serum or same anti-Hsp70 monoclonal antibodies that had been used to immunoprecipitate p70 or Hsp70, respectively. Bands were visualized as described above.

For Ii202 preclearing experiments, NIH3T3 cell lysates were first precleared as described above, followed by specific preclearing 2 times, for 1 hour and overnight, using 5 μl of anti-Ii202(SG) polyclonal antibody and 40 μl protein G-Sepharose. IP from precleared lysates was then performed with CD1 lupus serum or anti-Ii202(SG), using either the lysate precleared with anti-Ii202(SG) or a control lysate that was not precleared with specific antibody.

Two-dimensional phospho–amino acid analysis. Immunoprecipitates that had been electrophoresed and transferred to PVDF membranes were rinsed and exposed for autoradiography, and appropriate bands were excised. The radiolabeled bands were then subjected to acid hydrolysis as described previously (18), followed by 2-dimensional electrophoresis performed at 14°C.

RESULTS

Immunoprecipitation of a 70-kd and 50-kd protein from cell lysates using serum derived from CD1 lupus mice. Anti-CD1 TCR–transgenic T cells from donor BALB/c mice were transferred to irradiated BALB/c/nu/nu recipients, and development of lupus was monitored by measuring proteinuria and the presence of anti-dsDNA autoantibodies in the serum (8). To screen transgenic donor and adoptive recipient nu/nu mouse sera for additional autoantibodies, we performed IPs from lysates prepared from radiolabeled mouse cell lines. NIH3T3 fibroblasts and EL-4 lymphocytes were metabolically labeled with [35S]-methionine, and lysates were prepared. Sera obtained at day 45 from recipients of SP transgenic T cells (Figure 1, lane 3) and recipients of DN transgenic T cells (Figure 1, lane 4), as well as sera from donors of SP transgenic T cells (Figure 1, lane 2) and from an MRL++ mouse (Figure 1, lane 5) were used to precipitate autoantigens from the lysates. Immunoprecipitated proteins were separated by SDS-PAGE on 12% gels, transferred to nitrocellulose, and exposed for autoradiography.

Two striking patterns of antibody reactivities were observed in the sera from recipient mice with lupus that had been injected with SP transgenic T cells, but not in the sera from recipients of DN transgenic T cells without lupus. When sera from the SP transgenic T cell recipients were analyzed, one of the precipitated antigens migrated at ~70 kd (termed p70), and a second protein migrated as a broad, 50-kd band (termed p50) (Figure 1, lane 3). SDS-PAGE on 7% gels, which allows better resolution in the 50-kd range, revealed that the broad 50-kd band migrated as a distinct doublet of 48/52 kd (see Figure 5B, lane 3). Of the 4 animals of group A that received SP transgenic T cells and showed progression to severe lupus, 2 developed antibodies capable of precipitating p50 and p70 (Figure 1). The other 2 animals with lupus developed antibodies that recognized a novel RNP complex (19).

Serologic findings from both group A mice and group B mice and from control animals are summarized...
in Table 1. Of the 14 mice that were injected with the disease-inducing SP transgenic T cell subset, 9 developed severe GN, and 7 of those 9 animals had anti-p50 antibodies. No anti-p50 antibodies were found in the sera from the 5 animals that were injected with the disease-preventing DN T cells, from the 4 transgenic BALB/c donor animals, and from the 8 healthy control animals (5 normal BALB/c, 3 BALB/c/nu/nu). Anti-p70 autoantibodies were found in the sera from 5 of the 7 anti-p50-positive mice. Anti-p70 alone was also precipitated, albeit as a considerably weaker band, by serum from 2 of the SP transgenic T cell recipients that developed mild proteinuria but not overt lupus, by serum from 1 DN transgenic T cell recipient, and by serum from 1 transgenic healthy donor (Table 1). We conclude that anti-p50 antibodies are strongly associated with GN in the CD1 lupus-like model, and that a majority of CD1 lupus autoimmune sera coprecipitate p50 and p70.

Appearance of antibodies against p50 and p70 as early as 3 weeks after induction of disease. We sought to establish the time course of autoantibody development in the CD1 lupus-like model. CD1 lupus sera (from the recipients of SP transgenic T cells) were obtained at induction and at 5 time points after induction of disease. To minimize interassay variability, all sera were screened simultaneously for the presence of antibodies in a single IP experiment. Results from 2 representative animals with p50 and p70 reactivity are shown in Figure 2. In transgenic host animal A, antibody reactivity could be detected at day 20 after induction of lupus, and this peaked at day 35 and disappeared from day 45 onward. Animal A developed severe ascites between day 30 and day 45; the decline in antibody detection may thus have been caused by sequestration of Igs into the abdominal cavity. Animal B developed severe kidney disease but no significant ascites.

Table 1. Summary of serologic findings in the CD1 lupus model*

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of mice</th>
<th>Anti-p50</th>
<th>Anti-p70</th>
<th>Anti-p50 and anti-p70</th>
<th>Anti-dsDNA</th>
<th>Proteinuria</th>
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<tbody>
<tr>
<td>SP Tg recipient mice with severe kidney disease</td>
<td>9</td>
<td>7 (78)</td>
<td>5 (55)</td>
<td>5 (55)</td>
<td>9 (100)</td>
<td>Severe</td>
</tr>
<tr>
<td>SP Tg recipient mice with mild disease</td>
<td>5</td>
<td>0</td>
<td>2 (40)</td>
<td>0</td>
<td>1 (20)</td>
<td>Mild</td>
</tr>
<tr>
<td>DN Tg healthy recipient mice</td>
<td>5</td>
<td>0</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>Tg BALB/c healthy donor mice</td>
<td>4</td>
<td>0</td>
<td>1 (25)</td>
<td>0</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>Healthy BALB/c mice</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>Healthy BALB/c/nu/nu mice</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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</table>

* Except where indicated otherwise, values are the no. (%) with positive serologic findings. Anti-dsDNA = anti-double-stranded DNA; SP = single-positive (CD4+ or CD8+); Tg = (anti-CD1 T cell receptor) transgenic; DN = double-negative (CD4−,CD8−).
Categorizing p70 in the Hsp70 family. A spectrum of proteins belonging to the Hsp70 family migrates around 70 kd, as revealed by SDS-PAGE. Autoantibodies directed against Hsp70 were previously described in older MRL/lpr lupus mice (20). To investigate whether p70 might be a member of the Hsp70 family, we performed IP and WB experiments and used a mouse monoclonal antibody that specifically recognizes both constitutive and inducible Hsp70/Hsc70. First, we immunoprecipitated p70 with prototypic CD1 lupus serum and Hsp70 with anti-Hsp70 reference serum as a positive control, respectively. The nitrocellulose membrane containing the 70-kd proteins was then subjected to WB analysis with monoclonal antibody anti-Hsp70/Hsc70. A strong band was seen for both p70 (Figure 3A, lane 1) and Hsp70 (Figure 3A, lane 2), indicating that p70 is a member of the Hsp70 family.

To determine whether the CD1 lupus serum autoantibodies directly recognized Hsp70 on WB, we excised strips of nitrocellulose membrane containing p70 that were immunoprecipitated by 4 additional CD1 lupus sera, as well as Hsp70 that was immunoprecipitated by monoclonal antibody anti-Hsp70. In the subsequent WB step of the experiment, the strips were blocked and then incubated with 1:200 dilutions of the autologous serum (Figure 3B, lanes 1–4 and lane 6) or anti-Hsp70 (Figure 3B, lane 5). Bound serum antibodies were detected using HRP-conjugated donkey antimouse antibody, and visualized by chemiluminescence. All sera recognized a 70-kd band that exactly comigrated with Hsp70, suggesting that the autoantibodies directly recognize Hsp70 (Figure 3B, lanes 1–4). We were unable to unequivocally detect autoantibodies directly recognizing the p50 antigen, because of the presence of the comigrating Ig heavy chain (results not shown).

**Identification of p50 as a serine phosphoprotein.** We attempted to better characterize the 50-kgd antigen, first by investigating whether p50 was a phosphoprotein.

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**Figure 2.** Appearance of anti-p50/p70 antibodies as early as 20 days after induction of disease. Radiolabeled EL-4 cells were lysed and proteins were precipitated using serum obtained at sequential time points after disease induction (in days) from 2 individual anti-CD1 T cell receptor–transgenic (Tg) host mice, A and B. Along with the proteins, the relative migration of molecular mass markers is indicated on the left. Serum from an MRL++ mouse was used to precipitate U1 small nuclear RNP proteins, which are indicated on the right.

**Figure 3.** Identifying p70 as a member of the 70-kd heat-shock protein (HSP70) family of proteins. A, 35S-labeled proteins were immunoprecipitated using mouse antisera and anti-HSP70 reference immune serum, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 7% gel. Proteins were transferred to nitrocellulose membranes and exposed for autoradiography. The p70 bands were identified, and the membrane was excised accordingly. After overnight blocking, Western blotting with a 1:5,000 dilution of anti-HSP70/Hsc70 monoclonal antibody (mAb) was performed. B, p70 was immunoprecipitated using 4 different CD1 lupus sera (lanes 1–4) and anti-HSP70 mAb (lane 5). Individual membranes were blocked and then probed with 1:200 dilutions of the same sera and antibody, respectively, that were used for the immunoprecipitation. The relative migration of molecular markers is indicated with their relative molecular weights on the right.
EL-4 cells were metabolically labeled with $^{32}$P-orthophosphate for 5 hours, and after preparation of a cell lysate, we immunoprecipitated p50 and p70 with prototypic CD1 lupus sera. Proteins were subjected to SDS-PAGE, transferred to PVDF membrane, and exposed for autoradiography.

We found that p50 was precipitated not only from EL-4 lysates labeled with $^{35}$S, but also from lysates labeled with $^{32}$P, indicating that p50 is indeed a phosphoprotein (Figures 4A and B, lanes 1 and 3). In contrast, p70 was not precipitated from $^{32}$P-labeled protein extract, suggesting that this antigen is not phosphorylated (Figure 4B). The predominant site of phosphorylation was identified on serine residues, using 2-dimensional phospho–amino acid analysis (Figure 4C). Taken together, these results suggest that the p50 antigen is a 50-kd serine phosphoprotein that is coprecipitated along with an Hsp70 family member.

**Recognition of IFNα-inducible proteins by CD1 lupus serum.** Hsp70 has been identified by other investigators as an autoantigen in murine and human lupus (20,21), mixed connective tissue disease (22), and rheumatoid arthritis (23). Members of the Hsp70 family have been shown to associate with some members of the IFN-inducible Ifi200 proteins, and Hsp70 itself is inducible at an ~2-fold increased level in cells exposed to IFNα (24). In mice, Ifi200 proteins comprise Ifi202a and Ifi202b (~52 kd), Ifi203 (48 kd), Ifi204 (72 kd), and D3 (47 kd) (25,26). Ifi200 proteins recently have been identified as candidate lupus-susceptibility gene products in murine lupus (9). Biochemical characterization of p50 confirmed that molecular weight, phosphorylation, and association with Hsp70 are shared features of the unknown antigen p50 and several members of the Ifi200 family.

To elucidate the role of Ifi200 proteins as poten-
tial candidate antigens in the CD1 lupus-like model, we sought to determine whether p50 might be an IFN-inducible antigen. We used recombinant mouse IFNα to induce Ifi200 proteins in NIH3T3 cells. Cells were then metabolically labeled using 35S-methionine for 5 hours. After cell lysis, proteins were immunoprecipitated using 2 μl of mouse sera and anti-Ifi202 polyclonal antibodies. Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 7% gel. Arrows on the right indicate proteins precipitated by anti-Ifi202 immune serum, and arrows on the left indicate the antigens. In A, lanes 1–4 represent immunoprecipitations from protein extracts prepared from untreated 3T3 cells, and lanes 5–9 from 3T3 cells exposed to IFN-α. For CD1-transgenic (tg) host 3, only the result following IFN-α treatment is shown (lane 7). HSP 70 = 70-kd heat-shock protein. In B, 2 different anti-Ifi202 polyclonal antibodies (PL = Peter Lengyel, SC = Santa Cruz Biotechnology) were used to demonstrate precise comigration of IFN-α-inducible proteins recognized by anti-Ifi202 and a prototypic CD1 lupus serum, respectively. In C, lysate was either precleared twice with anti-Ifi202 (+) or not precleared (−). Proteins precleared from lysate (+) by anti-Ifi202 were run as a positive control (lane 5 in C). The strong unidentified band of −42 kd serves as a loading control.

Figure 5. Identifying p50 as an interferon-α (IFN-α)-inducible (Ifi) protein of the Ifi202 subfamily. NIH3T3 cells were separately incubated at 50% confluency without (−) or with (+) IFN-α for 18 hours, followed by metabolic labeling with 35S-methionine for 5 hours. After cell lysis, proteins were immunoprecipitated using 2 μl of mouse sera and anti-Ifi202 polyclonal antibodies. Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 7% gel. Arrows on the right indicate proteins precipitated by anti-Ifi202 immune serum, and arrows on the left indicate the antigens. In A, lanes 1–4 represent immunoprecipitations from protein extracts prepared from untreated 3T3 cells, and lanes 5–9 from 3T3 cells exposed to IFN-α. For CD1-transgenic (tg) host 3, only the result following IFN-α treatment is shown (lane 7). HSP 70 = 70-kd heat-shock protein. In B, 2 different anti-Ifi202 polyclonal antibodies (PL = Peter Lengyel, SC = Santa Cruz Biotechnology) were used to demonstrate precise comigration of IFN-α-inducible proteins recognized by anti-Ifi202 and a prototypic CD1 lupus serum, respectively. In C, lysate was either precleared twice with anti-Ifi202 (+) or not precleared (−). Proteins precleared from lysate (+) by anti-Ifi202 were run as a positive control (lane 5 in C). The strong unidentified band of −42 kd serves as a loading control.

Figure 5. Identifying p50 as an interferon-α (IFN-α)-inducible (Ifi) protein of the Ifi202 subfamily. NIH3T3 cells were separately incubated at 50% confluency without (−) or with (+) IFN-α for 18 hours, followed by metabolic labeling with 35S-methionine for 5 hours. After cell lysis, proteins were immunoprecipitated using 2 μl of mouse sera and anti-Ifi202 polyclonal antibodies. Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 7% gel. Arrows on the right indicate proteins precipitated by anti-Ifi202 immune serum, and arrows on the left indicate the antigens. In A, lanes 1–4 represent immunoprecipitations from protein extracts prepared from untreated 3T3 cells, and lanes 5–9 from 3T3 cells exposed to IFN-α. For CD1-transgenic (tg) host 3, only the result following IFN-α treatment is shown (lane 7). HSP 70 = 70-kd heat-shock protein. In B, 2 different anti-Ifi202 polyclonal antibodies (PL = Peter Lengyel, SC = Santa Cruz Biotechnology) were used to demonstrate precise comigration of IFN-α-inducible proteins recognized by anti-Ifi202 and a prototypic CD1 lupus serum, respectively. In C, lysate was either precleared twice with anti-Ifi202 (+) or not precleared (−). Proteins precleared from lysate (+) by anti-Ifi202 were run as a positive control (lane 5 in C). The strong unidentified band of −42 kd serves as a loading control.
and an Hsp70 family member, respectively (24). All CD1 lupus sera precipitated an IFN-inducible autoantigen, and this protein precisely comigrated with the 48-kd protein immunoprecipitated with anti-Ifi202 reference serum (Figure 5A, lanes 5–8). In additional experiments, both p48 and p52 were precipitated by prototypic CD1 lupus sera and precisely comigrated with the 48-kd and 52-kd proteins recognized by 2 different anti-Ifi202 polyclonal antibodies (anti-Ifi202[PL] and anti-Ifi202[SC]) (Figure 5B, and data not shown), suggesting that p50 is an IFN-inducible protein closely related to Ifi202 (see Discussion).

To confirm that anti-Ifi202 is recognized by CD1 lupus autoantibodies, lysates prepared from NIH3T3 cells were precleared twice with anti-Ifi202 antibodies. When the precleared lysate was used as a source of antigen, the 48-kd protein was no longer precipitated either by CD1 lupus serum (Figure 5C, lane 2) or by anti-Ifi202 antibodies (Figure 5C, lane 1). The protein precleared from the lysate was separated on the same gel (Figure 5C, lane 5), and precisely comigrated with the 48-kd proteins immunoprecipitated by CD1 lupus serum (Figure 5C, lane 4) and anti-Ifi202 (Figure 5C, lane 3). This result indicates that p50 and the 48-kd protein immunoprecipitated by anti-Ifi202 are identical or closely related. The higher 52-kd protein was not removed from the lysate (Figure 5C, lane 5), and correspondingly was detected by both CD1 lupus serum and anti-Ifi202 (Figure 5C, lanes 1 and 2).

**DISCUSSION**

In the present study, we discovered and characterized 2 autoantigens, p50 and p70, in the anti-CD1 TCR–transgenic lupus-like model. We identified p70 as a member of the Hsp70 family of heat-shock proteins. Biochemical and serologic characterization of p50 revealed that p50 is a member of the murine Ifi202-protein subfamily. Ifi202 family members have not yet been implicated as antigens in murine autoimmune disease, and therefore represent a truly novel class of autoantigens. Preliminary data indicate that IFNα-inducible proteins are targeted by humoral autoimmune responses in older MRL++ and MRL/lpr mice, providing further evidence that this class of autoantigens may play a role in murine lupus (results not shown). These findings are consistent with the results in earlier reports which demonstrated autoreactivity against the IFNγ- and IFNα-inducible Ifi16 protein in subsets of lupus patients (15).

Ifi200 proteins are encoded on mouse chromosome 1 within the Ifi200 gene cluster and are important regulators of cell growth and differentiation (14). Ifi202 is a key inhibitor of various transcription factors and associates with a 68-kd member of the Hsp70 family (24). This study showed that Ifi202 proteins from 3T3 cells were undetectable on WB analysis, unless the proteins were first induced by activation of cells with IFNα, but were weakly detectable by IP from radiolabeled cell lysates. The fact that Ifi202 proteins are expressed at low levels in some cells may explain why our initial experiments using uninduced cells (Figures 1–4) identified an autoantigen by IP. Herein we show that p50 shares several biochemical features with Ifi202: molecular weight, phosphorylation, IFNα inducibility, and association with Hsp70 (24). Choubey and Lengyel previously demonstrated that anti-Ifi202 polyclonal antibodies coprecipitate 3 proteins of 68 kd, 52 kd, and 48 kd, that the 48-kd and 52-kd bands precisely comigrate with in vitro–translated Ifi202, and that only the 52-kd Ifi202 band is recognized by anti-Ifi202 polyclonal antibodies on WB analysis (24). Since both of the anti-Ifi202 polyclonal antibodies used in our studies are not known to cross-react with Ifi203, it is most likely that the lower molecular weight protein seen in our experiments represents a posttranslational modification of Ifi202, such as a phosphorylated isoform.

Ifi202 exists as 3 variants, Ifi202a, Ifi202b, and Ifi202c (25). Our observation that the 52-kd factor could not be cleared from the lysates by anti-Ifi202 may be explained by the existence of 2 different Ifi202 complexes, whereby only epitopes in one complex are accessible to both CD1 lupus antibodies and anti-Ifi202 antibodies. Taken together, our results suggest that p50 is an Ifi202 isoform or a homolog within the Ifi202 subfamily of proteins.

Ifi202 from the Ifi200 gene cluster has recently been identified among ~500 genes on mouse chromosome 1 to be a dominant gene locus controlling susceptibility for lupus outside the MHC system (9). It is striking that autoantibodies produced in the CD1 lupus-like model are directed against proteins encoded by the Ifi202 gene locus. The functional significance of this observation, and a potential pathogenic role of autoantibodies directed against IFN-inducible antigens, awaits further experimentation (11). In a hypothetical model, intermittent or chronically sustained increases of IFNα concentrations activate IFN-inducible genes. We are not aware of investigations that implicate increased expression of Ifi202 protein or Hsp70 in the severity of lupus. In a recent study of SLE patients, transcriptional profil-
Recent studies suggest a pivotal role for IFN-α in the pathogenicity of SLE. Notably, several features of current pathogenic models of lupus, such as infections, ultraviolet light, or other stressors, are prominent factors that activate the IFN system, such as infections, ultraviolet light, or other stressors, are prominent factors that activate the IFN system. Moreover, many exogenous factors that activate the IFN system, such as infections, ultraviolet light, or other stressors, are prominent factors that activate the IFN system. Furthermore, recent studies suggest a pivotal role for IFN-α in SLE initiation by virtue of its ability to induce activation and differentiation of dendritic cells (11), and in the perpetuation of disease by providing a positive feedback loop involving endogenous IFN-α-induced DNA/anti-DNA immune complexes (12,30). Finally, autoantibodies, and more rarely autoimmune syndromes, are occasionally induced in patients treated with IFN-α for chronic hepatitis B and hepatitis C infection, and in patients with several malignant disorders (for review, see ref. 32). In this context, SLE is the most frequently encountered IFN-α-induced autoimmune disease.

In contrast, the role of IFN-α in murine lupus is much less rigorously investigated. A recent report demonstrated that disruption of IFN-α signaling in NZB mice by deleting type I IFN receptors significantly decreased the spontaneous development of lupus in these mice (13). Other investigators reported that the enzyme RNA helicase A is an autoantigen in NZB/NZW mice (33), and genetic analysis suggested that the gene encoding RNA helicase A is likely to be regulated by IFNs (34). In a recent murine lupus study using the B6/lpr background, higher autoantibody levels, enhanced immune complex formation, and more severe kidney disease were reported following sustained induction of type I IFNs (35). In contrast, we did not observe autoantibodies against inducible antigens in a group of NZB/NZW mice (results not shown). However, due to the small sample size in our study, we cannot rule out the possibility of autoreactivity directed against IFN-α-inducible proteins in hereditary lupus. In other preliminary experiments, we found that a subset of autoimmune sera from older MRL++ and MRL/lpr mice precipitated IFN-α-inducible proteins (results not shown).

Future studies will be directed toward the broader characterization of autoreactivity against IFN-α-inducible proteins, using larger panels of sera from these models of lupus. Moreover, the identification of inducible antigens and the elucidation of a potential link between IFN-driven overexpression of inducible protein antigens and clinical disease are important issues to address. Since IFN biosignatures, as determined by transcriptional profiling, have recently been reported to identify patients with higher disease scores, in terms of the presence of the SLE classification criteria and more severe disease (27), it will be interesting to analyze whether profiles of autoantibodies directed against IFN-α-inducible proteins are associated with such IFN biosignatures.

Hsp70 is a known autoantigen in murine lupus (20) and human SLE (21). Gsp78, a member of the Hsp70 family, has recently been implicated as a target in...
early autoimmune responses in a murine lupus model, and an intimate association with the lupus autoantigens Ro52 and Ro60 has been demonstrated (36). However, anti-Hsp70 antibodies are also detectable in a variety of autoimmune diseases other than lupus, including rheumatoid arthritis (23). Although anti-Hsp70 antibodies have been suggested as markers for mixed connective tissue disease (22), the principal diagnostic value of anti-Hsp70 antibodies in autoimmune diseases remains unclear (for review, see ref. 37).

Is there a role for CD1 in the development of lupus and in breaking tolerance to certain antigens? CD1 has been implicated in both murine (7) and human (38) SLE, and may be a target for therapeutic interventions (7). Moreover, our finding of protein antigens in the anti-CD1 TCR–transgenic lupus-like model suggests that autoantigens derived from protein complexes may be presented by CD1. The therapeutic potential of such putative ligands would be tremendous and certainly warrants further investigation.

In summary, IFN-α-inducible antigens represent a new class of inducible autoantigens. We speculate that adjustments of current immunoassays to enable detection of these novel cytokine-inducible antigens may considerably extend our knowledge of the role of autoantigens in lupus and other autoimmune diseases.

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