when Northern blots containing RNA from NIT-1 and βTC1 cells were hybridized with the Ham-2–specific probe, equivalent induction of Ham-2 transcripts was observed. Thus, the rare NOD Ham-1d and Ham-2d alleles carried in the NIT-1 cell line did not inhibit transcriptional induction by IFN-γ. HAM1 protein expression was also induced in β cells in response to IFN-γ (Fig. 4A). A single band of approximately 65 kD corresponding to HAM1 was detected only in cell lysates from IFN-γ–treated cultures. A comparable IFN-γ inducive effect on HAM1 protein was observed in βTC3 and NIT-1 cell cultures (Fig. 4B).

Two putative ATP-binding cassettes are present in the HAM1 protein sequence (3); however, HAM1 affinity for ATP has not been reported. We used an ATP-agarose purification technique (6) to compare the ATP affinity of HAM1 to that of a known high affinity ATP-binding protein, heat shock protein 70 (hsp70). As expected, the constitutive hsp70 protein remained bound to the ATP-agarose even after extensive washing with high salt buffer (2 M NaCl), which demonstrated the high ATP affinity of this molecule (Fig. 4B). In contrast, the HAM1 protein was detected only in the unbound lysate, which indicated low or no affinity for ATP under the assay conditions (Fig. 4B).

These results contrast with Faustman et al.’s conclusion that the Xba I RFLV associated with the NOD Ham-1b allele represented a mutation that impaired gene function (2). Faustman et al. further propose that reduced Ham RNA expression is contributable to MHC-linked autoimmune diabetes susceptibility on the basis of their observation of reduced cell surface expression of MHC class I molecules on NOD splenocytes. However, our results show normal constitutive and cytokine-regulated expression of Ham-1 in NOD/Lt peritoneal macrophage cultures. In addition, although cultured β cells (including the NIT-1 cell line and primary islet cultures) differed from macrophages in that they did not exhibit constitutive Ham-1 expression, we found that this expression was IFN-γ–inducible and that the response was not impaired in NIT-1 cells. Finally, the fact that IFN-γ treatment of a NOD/Lt β cell line produced HAM1 molecules of correct molecular size confirmed that Ham-1 mRNA was translated. Consistent with normal regulation of Ham loci, we observed nominal levels of MHC class I antigen on the cell surfaces of freshly isolated splenic leukocytes from prediabetic NOD/Lt mice (7). It is generally accepted that RFLVs only denote differences in restriction enzyme sites in homologous DNA regions. Thus, in the absence of precise comparison of gene sequences to establish the relation of unusual Ham-1 restriction endonuclease sites to transcribed regions, altered fragments sizes should not necessarily be considered synonymous with function-abrogating mutations in coding or regulatory regions.

Both the Ham-1 and Ham-2 genes are required for antigen processing, as transcription of these genes into processing–degrading cells restores antigen processing capability (8). Although our results, which we obtained with cells in vitro, demonstrate normal Ham-1 gene expression, HAM function in NOD mice could nonetheless be abnormal. For example, the Ham genes within the H-2d7 haplotype could contribute to diabetes susceptibility by influencing the repertoire of peptides available for presentation by MHC class I molecules. Altered ATP-binding affinities for NOD Ham gene products may also exist. However, we were unable to demonstrate an ATP affinity for NOD or control strain–derived HAM1 proteins, so the lack of HAM1 binding may necessitate a reconsideration of the hypothesis that HAM molecules function as ATP-dependent peptide pumps (3), which is consistent with a recent report (9).

Extended haplotype analysis of H-2k7 continues to reveal the presence of other unique alleles that may also contribute to the diabetogenic potential of this haplotype (10–12). In addition, many intra-MHC genes required for distinct aspects of antigen processing are now being revealed (4, 13, 14). We propose that detailed investigation of the structure and regulated expression of these genes in the diabetogenic H-2k7 haplotype will finally link genetic regions and immunodysfunctions that characterize autoimmune diabetes in the NOD mouse.

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REFERENCES AND NOTES

1. A recent HLA Nomenclature Committee meeting resolved that the names Tap-1 and Tap-2 (for transporter associated with antigen processing) be used for Ham-1 and Ham-2 and for their homologs in rats and humans (J. J. Monaco, Immunol. Today 13, 173 [1992]).


5. The pancreatic β cell lines, βTC1 and βTC3 brought kindly provided by S. Efrat (Albert Einstein Col-lege of Medicine, Bronx, NY) originated from β cell adenomas in transgenic mice segregating C57BL/6J and DBA/2J genome (D. Hanahan, Nature 315, 115 [1985]); the H-2 haplotype of βTC1 is H-2b/H-2k, whereas the βTC3 line is homozygous H-2b. The NIT-1 β cell line originated from a β cell adenoma in an NOD/Lt transgenic mouse (K. Hamaguchi, H. R. Gaskins, E. H. Leiter, Diabetes 40, 842 [1991]).


19. The technical assistance of H. Chapman is gratefully acknowledged. We thank D. Serreze and P. Schwartz for constructive review of the manuscript. This work was supported in part by NIH grants DK 38175 (E.H.L.), DK 27272 (E.H.L.), AI 32761 (J.J.M.), and by fellowships from the Juvenile Diabetes Foundation, International (H.R.G.), and the American Diabetes Association (H.R.G.).

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Faustman et al. propose that faulty expression of major histocompatibility complex (MHC) class I molecules is linked to autoimmune diabetes. They found (1) decreased class I expression in both 6-week-old normoglycemic and 27-week-old hyperglycemic nonobese diabetic (NOD) mice as compared with that in BALB/c mice or in 27-week-old normoglycemic NOD mice. Two of the monoclonal antibodies (MAB) used by Faustman et al. were SFI-1.1 (PharMingen, San Diego, California) and 9010A (Accurate Chemical, Westbury, New York), both of which react with the class I product Kd.

Our studies of the NOD mouse model of type 1 diabetes have used NOD/MkrTac mice, which develop a high incidence of diabetes when housed in our facility (>80% of females and 50% of males by 7 months of age) (2). The NOD mouse, which has the H-2k7 MHC haplotype, expresses the cell surface antigens Kd, I-Ak7, and Dk. We have identified one of the class I products of the NOD mouse with SFI-1.1, a MAB of the IgG2a isotype reported to react with Kd but not the H-2b, I-Ak, or Dk haplotypes (3). We have also used MAB SFI-1.1 to develop two reciprocal MHC congenic strains of mice: B10.H-2k7 (2) and NOD.H-2b (4). As expected, B10.H-2k7 spleen cells, but not NOD.H-2b spleen cells, were
Table 1. MHC Class I expression on splenic T and B cell subpopulations. MFI, mean fluorescence intensity of MHC class I expression of the cells in the subpopulation. Spleen cells were incubated with phycoerythrin-labeled mononuclear antibodies, which recognize the T cell surface markers CD4 (clone RM-4-5, PharMingen) and CD8 (clone S3-6.7, PharMingen) or which recognize the B cell surface marker B220 (clone ODN-1-9, PharMingen). Spleen cells were incubated in a volume of 0.1 ml with 2 µg of antibody to CD4, 0.4 µg of antibody to CD8, antibody to CD4 and antibody to CD8 together, or 0.24 µg of antibody to B220. The cells were incubated with the phycoerythrin-labeled antibodies for 20 min at 4°C and then with fluorescent-labeled antibody to Kd, as described in Fig. 1. The mean fluorescence intensity of class I expression in each subpopulation was obtained by gating on phycoerythrin-positive cells.

<table>
<thead>
<tr>
<th>Mouse strain and age</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4/CD8 B220</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c 25 weeks NOD</td>
<td>19.5</td>
<td>7.2</td>
<td>27.1 65.0</td>
</tr>
<tr>
<td>6 weeks NOD</td>
<td>35.3</td>
<td>12.5</td>
<td>45.6 43.9</td>
</tr>
<tr>
<td>16 weeks NOD</td>
<td>32.2</td>
<td>14.1</td>
<td>45.6 44.2</td>
</tr>
</tbody>
</table>

Fig. 1. Dissociated spleen cells (10%) depleted of red blood cells, were incubated with 0.53 µg of fluorescein-labeled murine MAb SF1-1.1 (anti-Kd, PharMingen) or 2 µg of fluorescein-labeled murine MAb AF6-88.5 (anti-Kd, PharMingen). Saturating amounts of both antibodies were used. After incubation with the antibodies for 20 min at 4°C, the cells were washed and analyzed by flow cytometry on the FACStarPLUS (Becton-Dickinson, Mountainview, California). Propidium iodide was added to eliminate dead cells. Mice were purchased from Taconic Farms (Germantown, New York) and housed under sterile, specific pathogen-free conditions. The BALB/c and C57BL/6 mice were 17 and 9 weeks of age, respectively. All mice were female except for the diabetic NOD mouse.

Fig. 2. Class I expression on splenic T and B cell subpopulations. Before incubation with fluorescent-labeled antibody to Kd, spleen cells (10%) were incubated with phycoerythrin-labeled antibody to CD4 and antibody to CD8 (added together) to identify T cells or with phycoerythrin-labeled antibody to B220 to identify B cells (see Table 1 for antibody concentrations). In (A) to (C), class I expression on all spleen cells from the mice indicated is compared with that on CD4+/CD8+ cells within the spleen cell population (darkened profile in each panel). In (D) to (F) class I expression on all spleen cells is compared with class I expression on B220+ cells (darkened profiles). The mean fluorescence intensity of class I expression on each cell subpopulation was obtained by gating on phycoerythrin-positive cells. The data in Table 1 and Fig. 2 are from the same experiment.

1. Therefore we examined class I expression on splenic T and B cell subpopulations by two-color fluorescence analysis (Table 1; Fig. 2). Both BALB/c and NOD B220+ B cells expressed 30 to 90% higher amounts of class I antigen expression than either CD4+ or CD8+ T cells. Because NOD mice have a higher percentage of T cells in their spleen compared with BALB/c mice (45.6 as opposed to 27.1%) (Table 1), a higher proportion of NOD spleen cells would be expected to express lower amounts of class I antigen as compared with BALB/c spleen cells.

Class I expression on NOD T cells is slightly lower (10 to 20%) than on BALB/c T cells, whereas class I expression on B cells from 6-week-old NOD mice is nearly identical to that on BALB/c B cells (Table 1). By 16 weeks of age, however, the B cells from NOD mice exhibit 40% more class I expression than B cells from 6-week-old NOD mice (Table 2). This increase in B cell class I expression produces the prominent bimodal distribution observed in spleen cells obtained from NOD mice greater than 6 weeks of age (Figs. 1 and 2).
phenotyping on NOD splenocytes with a directly conjugated MHC class I MAb SF1-1.1 (PharMingen, San Diego, California), and demonstrated decreased background cross-reactivity with negative C57BL/6 splenocytes. Consistent with standard immunological procedures, their data show that the high background immunofluorescence of mouse splenocytes, observed with the use of FITC-conjugated goat antibodies to mouse immunoglobulin, can be eliminated with MAb SF1-1.1. When we used directly conjugated MAb SF1-1.1, we observed MHC class I data identical to that obtained by Wicker et al. As expected, we also observed decreased cross-reactivity with C57BL/6 splenocytes. Nevertheless, we observed a diminished MHC class I density with both techniques in the NOD mouse.

In humans with diabetes, lymphocytes show consistent definitive expression of MHC class I antigens. However, in NOD mice splenocytes, different antibodies to MHC class I antigens show varying degrees of defective MHC class I expression. The 9008-A antibody to H-2K, which binds to the epitope binding region of the K molecule, has shown a more striking defect in MHC class I expression in NOD splenocytes than has MAB SF1-1.1 (Fig. 2A).

We prospectively monitored 201 females in our colony of NOD mice with a high incidence of diabetes from 4 to 6 weeks of age with 9008-A antibody to test the predictive power of this change in MHC class I reactivity with regard to disease penetrance. We found by flow cytometric analysis of 169 mice that MHC class I density decreased on peripheral blood lymphocytes, as reported by Gaskins et al. (Fig. 2A); the remaining 32 mice showed MHC class I expression patterns identical to those of control BALB/c mice (Fig. 2B). By 9 months of age, 100% of the 169 mice that showed modified MHC class I expression developed severe hyperglycemia and died. At 18 months of age, the 32 mice with normal lymphocyte cell surface expression of MHC class I remained normoglycemic. These results are similar to our data for human identical twins discordant for type I diabetes (1). Therefore, diminished expression of MHC class I antigens observed on the surface of peripheral lymphocytes is predictive of the progression to hyperglycemia in genetically susceptible populations of both humans and mice.

Another important technical point is that exposure of murine splenocytes to serum-containing media at room temperature...

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Note: The provided text is a summary of the findings and methodology described in the original scientific paper. The full text of the paper provides a more detailed and comprehensive explanation of the experiments and results.
Expression of intra-MHC transporter (Ham) genes and class I antigens in diabetes-susceptible NOD mice
LS Wicker, PL Podolin, P Fischer, A Sirotina, RC Boltz Jr and LB Peterson

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