Differential Regulation of Constitutive Major Histocompatibility Complex Class I Expression in T and B Lymphocytes

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Summary

Major histocompatibility complex (MHC) class I antigens are constitutively expressed yet highly induced by interferon (IFN) during inflammation. We found that not only IFN-induced but also normal basal expression of MHC I required IFN receptors and signal transducer and activator of transcription (STAT)1, providing genetic evidence for continuous IFN signaling. Surprisingly, an IFN-independent requirement for STAT1 was also found, specifically in T lymphocytes, where MHC class I expression was not fully accounted for by IFN signaling. This IFN-independent pathway maintained tyrosine phosphorylation of STAT1 in T but not B lymphocytes even in the absence of IFN receptors. Interestingly, interleukin (IL)-7 selectively activated STAT1 and induced MHC class I in mature T but not B cells. These loss of function studies demonstrate an essential role of endogenous IFN and activated STAT1 for constitutive MHC class I expression in normal mice and define IL-7–dependent but IFN-independent regulation of STAT1 restricted to T lymphocytes.

Key words: interferon • STAT1 • interleukin-7 • gene regulation • tyrosine phosphorylation

MHC class I antigens, which are cell surface glycoproteins composed of a heavy chain and a light chain (β2-microglobulin [β2m]), are ubiquitously expressed on most nucleated cells. The levels of MHC class I can be regulated by various cytokines, including IFNs and TNF-α, and by mitogens such as LPS, phorbol esters, and Con A (1). Expression of MHC class I is tightly regulated during development (2–4) and differentiation (5) and is required for immune recognition of virus infection and for T cell development. Antigen presentation by MHC class I is essential for both the development and the effector function of CD8^{+} CTLs. Differential expression of MHC class I on thymic epithelial and dendritic cells influences both positive and negative selection of immature thymocytes (6).

Expression of MHC class I genes is controlled by both transcriptional and posttranslational mechanisms. Transcriptional regulation of MHC class I heavy chain is controlled through transcription factor binding sites in the promoters of the MHC class I genes (7, 8). Conserved cis-acting elements include enhancer A, IFN consensus sequence (ICS),[1] and site α. Enhancer A contains regions I and II recognized

by members of the retinoic acid receptor family (5) and the nuclear factor (NF)-κB/Rel family (9), respectively. The binding of NF-κB is essential for constitutive class I expression and for induction by mitogens and by cytokines such as TNF (10). ICS contains an IFN-stimulated response element that is the target site for transcription factors of the IFN regulatory factor (IRF) family, such as IRF-1, IRF-2, IRF-9 (ISGF3γ p48), and IRF-8 (ICS-binding protein), and it mediates the induction of MHC class I expression by IFNs. The interactions of different transcription factors at enhancer A and ICS sites can achieve synergistic effects on the regulation of MHC class I genes (11). MHC class II transactivator (CIITA), a global regulator for MHC class II molecules, is also involved in the constitutive and IFN-γ–induced expression of MHC class I genes through site α (12, 13).

Two sets of molecules involved in antigen processing and presentation are also essential for cell surface expression of MHC class I molecules (14, 15). LMP2 and LMP7, two subunits associated with the 20S proteasome, are required for enhanced processing of cytosolic proteins into small peptides (16). Mice lacking LMP2 and LMP7 are deficient in presenting antigens and in MHC class I surface expression (17, 18). Transporter associated with antigen processing (TAP)1 and TAP2 are transporter proteins that form heterodimers and facilitate movement of peptides from the cytosol into the lumen of the endoplasmic reticulum, where

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[1]*Abbreviations used in this paper:* BMT, bone marrow transplantation; DN, double-negative; DP, double-positive; EMSA, electrophoretic mobility gel shift; GFP, green fluorescent protein; ICS, IFN consensus sequence; IRF, IFN regulatory factor; NF, nuclear factor; PML, promyelocytic leukemia; SP, single-positive; STAT, signal transducer and activator of transcription; TAP, transporter associated with antigen processing.

they are loaded into the groove of the MHC class I heavy chain, and these transporters are also required for MHC class I expression (19). For instance, RMA-S, a mutant cell line lacking TAP2, is defective in the expression of MHC class I on the cell surface owing to its rapid degradation in the absence of peptide (20). MHC class I structural genes as well as the processing machinery and some of the regulatory transcription factors are all induced by IFN during inflammation.

Induction of the MHC complex by type I and type II IFN is abrogated in the absence of signal transducer and activator of transcription (STAT)1 (21, 22), demonstrating the absolute requirement of STAT1 for IFN-stimulated transcriptional responses. As no STAT1 binding site was found in the promoter of the MHC class I gene, the role of STAT1 in IFN-γ–mediated MHC class I induction is likely to be indirect. Several transcription factors implicated in MHC class I expression are themselves targets for STAT1, such as IRF-1 and CIITA. However, during IFN-α/β responses, STAT1 may directly induce class I gene expression by forming the ISGF3 complex in conjunction with STAT2 and IRF-9, which in turn binds to the ICS of MHC class I promoters (23). The fact that IFN-β was capable of inducing MHC class I expression in IRF-1$^{-/-}$ fibroblasts suggests that the binding of ISGF3 to the ICS is sufficient for MHC class I induction (24).

JAK–STAT signaling, first characterized in the IFN system, has been shown to be essential for many cytokine responses (25, 26). One of the surprises revealed by genetic studies of STAT deficiency is the remarkable specificity of individual STAT molecules for distinct cytokine signaling systems (27) in spite of the more promiscuous activation observed in cell culture. However, we have also explored possible roles for STAT1 outside of inflammatory responses to IFN. For instance, a role for STAT1 in response to fibroblast growth factor was detected during chondrocyte development in organ cultures (28), and in this paper we provide evidence that STAT1 is required for basal expression of MHC class I. Part of this requirement for STAT1 is due to a constitutive rather than an inflammatory role for IFN and demonstrates a steady-state requirement for IFN in the absence of infection. Unexpectedly, we also observed an IFN-independent and cell type–restricted role for STAT1 specifically in T but not B lymphocytes.

The cell type specificity of the IFN-independent STAT1 requirement corresponds to the responsiveness of cells to IL-7. IL-7 is a cytokine that is essential for lymphoid development (29–31), with its specific receptor IL-7Rα expressed on T and B lineage progenitors. In the thymus, double-negative (DN) and single-positive (SP) cells but not double-positive (DP) cells express IL-7Rα, and the receptor is expressed on peripheral T but not B lymphocytes. IL-7 signals through JAK1 and JAK3 and has been shown to activate STAT5a and STAT5b (32) and possibly STAT1 (33), and it promotes the survival of lymphoid cells, which is particularly critical for thymic precursors (34). Recently, IL-7 was also shown to induce immune recognition molecules, including MHC class I, in acute myeloid leukemia

and lymphoid leukemia patients (35). Here, we show that IL-7 also activates STAT1 in mature T lymphocytes, leading to increased expression of MHC class I.

Materials and Methods

Animals. Generation of STAT1$^{-/-}$ (36), IFN-α receptor (IFNAR)1$^{-/-}$ (37), and IFN-γ receptor (IFNGR)1$^{-/-}$ mice (38) has been described. IFNAR and IFNGR double-knockout mice (AR+GR)$^{-/-}$ were derived by interbreeding IFNAR$^{-/-}$ and IFNGR$^{-/-}$ mice and screening for compound homozygous mutant offspring. Strain backgrounds were either 129 or C57BL/6 (eighth backcross generation) and were compared with wild-type mice of the same strain. Mice were housed under specific pathogen–free conditions, and all work with animals conformed to guidelines approved by the Institutional Animal Care and Use Committee of New York University School of Medicine.

Flow Cytometry. Lymphocytes of thymi, spleens, and lymph nodes were prepared from 6–8-wk-old mice. Triple staining for surface markers was performed by incubating ∼10^6 cells first with M1/42.3 (TIB-126; American Type Culture Collection), a rat mAb that recognizes a framework epitope common to mouse MHC class I, followed by staining cells with FITC-conjugated anti–rat IgG (Caltag Labs.). Cells were subsequently incubated with anti–CD4-TC (tricolor) and anti–CD8-PE or anti-B220-PE antibodies (Caltag Labs.) in the presence of normal rat serum to block cross-reactions. Stained cells were washed two times with cold staining buffer (0.2% BSA and 0.1% sodium azide in PBS) and fixed with 1% paraformaldehyde in staining buffer, followed by FACS™ analysis. Additional surface markers were analyzed using anti-H-2K^b–PE (PharMingen) and anti–I-A^b–biotin, anti–IgM-biotin, and streptavidin–allophycocyanin (Caltag Labs.). For comparing MHC class I induction in response to IFN-γ and IL-7, selected lymphocyte populations were incubated in the presence or absence of cytokine for 48 h, followed by FACS™ analysis. Mean channel shift was the average increase in mean fluorescence intensity (39) and varied <10% for three separate experiments.

Northern Blot Analysis. 20 μg of total RNA prepared from splenocytes or thymocytes using Trizol reagent (Life Technologies) was resolved on 1.5% denaturing agarose gels and transferred to nitrocellulose membranes. cDNA probes were derived from splenic RNA by reverse transcriptase–PCR. The primer sets for amplifying different genes were as follows: LMP2, forward primer, 5′-ATGCTGCGGGCAGGAGCACCTACCGC-3′ and reverse primer, 5′-TCACTCATCGTAGAATTTTTGGCAGCTC-3′; LMP7, forward primer, 5′-ATGGCGTTACTGGATCTGTGCG-GTGC-3′ and reverse primer, 5′-TCACAGAGCGGCCTCTC-CGTACTTGTA-3′; TAP1, forward primer, 5′-AGTGTCTC-GGGAATGCTGCTGAAGGTG-3′ and reverse primer, 5′-AGT-GTGCAGTCCAGAGGCCTTGTCGTCTG-3′; β-actin, forward primer, 5′-GTGGGGCGCCCCAGGCACCA-3′ and reverse primer, 5′-CTCCTTATTGTCACGCACGATTTC-3′; MHC I, forward primer, 5′-GCACAGATTCCCCCAAAGG-3′ and reverse primer, 5′-ATCTCAGGGTGAGGGGCTCA-3′; β2m, forward primer, 5′-GTGACCCTAGTCTTTCTGGTG-3′ and reverse primer, 5′-TGAATCTTCAGAGCATCATG-3′; IRF-1, forward primer, 5′-TCCATGGAAGCACGCTGCTA-3′ and reverse primer, 5′-AGACTGCTGCTGACGACACA-3′.

The amplified DNA fragments were purified and labeled using RadPrime DNA labeling kits (Life Technologies). Hybridization, washing, and stripping of the nitrocellulose membrane followed standard procedures (40). To quantify specific signals, membranes were exposed to PhosphorImager screens (Molecular Dynamics)

and analyzed according to the manufacturer's instructions. Quantitative measurements were normalized to values for actin.

Retroviral Transduction of Bone Marrow Cells and Bone Marrow Transplantation. Production of retrovirus was as described (41). A murine stem cell virus vector expressing humanized green fluorescent protein (GFP; a gift of Dr. W. Pear, University of Pennsylvania, Philadelphia, PA) was transfected into the Phoenix packaging cell line (a gift of Dr. G.P. Nolan, Stanford, CA), and culture supernatant was used as viral stock. Transduction of bone marrow cells with retrovirus was as described (42). In brief, C57BL/6 wild-type or STAT1$^{-/-}$ mice were treated with 150 mg/kg 5-fluorouracil (Sigma Chemical Co.) 6 d before harvest. Bone marrow cells were harvested and incubated on Retronectin™ (Takara-Shuzo) coated dishes containing bone marrow transfer medium (IL-3, 6 ng/ml; stem cell factor, 10 ng/ml; and IL-6, 10 ng/ml, all from PeproTech, Inc., and 20% FBS in RPMI 1640) and 1 ml of recombinant virus for 48 h. Cultures were supplemented with 1 ml of fresh virus after the first 24 h. 1.6 million bone marrow cells were injected intravenously into lethally irradiated (900 rads) wild-type or STAT1$^{-/-}$ recipients. Mononuclear cells recovered from peripheral blood of recipient mice were analyzed by double staining with anti-H-2K^b-PE and anti–TCR-allophycocyanin or IgM-allophycocyanin after gating for GFP fluorescence to score donor-derived lymphocytes.

Separation of T and B Lymphocytes and Analysis of Nuclear Extracts. Freshly isolated splenocytes were incubated with rat anti-B220 at 0.2 μg/10^6 cells for 30 min at 4°C. Antibody-bound cells were washed twice with 1× PBS and incubated with anti–rat antibody–coated M450 Dynabeads (Dynal) for 30 min at 4°C on a mixer. B cells were collected using a magnet after three washes with 1× PBS containing 0.2% BSA. Nuclear extracts prepared from positively selected B cells and negatively selected T cells were resolved in 7% SDS-PAGE, followed by Western blot analysis as described (43). The antibodies used for immunoblots were anti–phosphotyrosyl-STAT1 (Zymed Labs.) and anti–STAT1 COOH terminus (STAT1C; a gift from Dr. C. Schindler, Columbia University, New York, NY). Purity of selected cell populations was verified by flow cytometry. B cell fractions were typically >95% pure; T cell fractions were >80% pure.

Electrophoretic Mobility Gel Shift. Nuclear extracts prepared from Dynabead-selected T or B cells, which were left untreated or treated with human recombinant IL-7 (10 ng/ml; StemCell) or IFN-γ (100 U/ml; Boehringer Mannheim) for 30 min, were analyzed using a ^{32}P-labeled probe containing a high-affinity GAS sequence (44) in the presence or absence of anti-STAT1C antibody as described (43).

Results

Decreased Surface Expression of MHC Class I on STAT1$^{-/-}$ Lymphocytes. Type I and type II IFNs are known modulators of MHC antigen expression during inflammation and viral infection (45). Because STAT1 is a signal transducer shared by both types of IFNs, we examined the expression of MHC antigens on freshly isolated lymphocytes from wild-type or STAT1$^{-/-}$ mice with antibody to either MHC class I (M1/42.3, an mAb recognizing a framework epitope of MHC class I) or class II (I-A^b). Cells from STAT1$^{-/-}$ mice housed under pathogen-free conditions showed a two- to threefold reduction of surface MHC class I compared with lymphocytes from wild-type mice (Fig. 1 A). The expression of MHC class II, however, was comparable
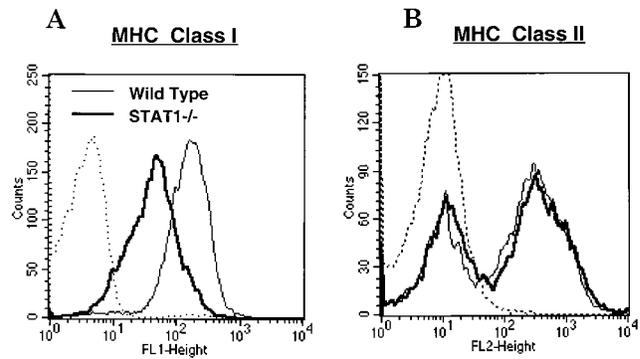

Figure 1. Reduced surface expression of MHC class I, but not class II, on STAT1$^{-/-}$ lymphocytes. Freshly isolated splenocytes prepared from wild-type (thin lines) or STAT1$^{-/-}$ mice (bold lines) were stained with antibodies against MHC class I (A) or class II (anti–I-A^b) (B), followed by FACS™ analysis. Dotted/dashed lines represent staining with secondary antibody alone.

on lymphocytes of both genotypes (Fig. 1 B), even though both class I and class II expression can be induced by IFN, and both show an absolute requirement for STAT1 for induction (21). Reduced expression and absence of induction of MHC class I was not due to a global defect, however, because class I expression was induced in response to mitogenic stimulation of both B and T splenocytes devoid of STAT1 (data not shown), reflecting an intact NF-κB pathway (46–48).

Reduced Expression of mRNA for MHC Class I Genes and Antigen Processing and Presentation Molecules in STAT1$^{-/-}$ Lymphocytes. To explore the mechanisms underlying the reduction of cell surface expression of MHC class I in the absence of STAT1, we examined the levels of MHC class I heavy chain and light chain (β2m) mRNA by Northern hybridization. To assess the potential role of constitutive IFN in this regulation, we included lymphocytes lacking receptors for either IFN-α or IFN-γ derived from IFNAR$^{-/-}$ or IFNGR$^{-/-}$ mice. As shown in Fig. 2 A, MHC class I heavy chain and β2m mRNA levels were reduced significantly in both thymi and spleens of STAT1$^{-/-}$ mice. Quantitation of the mRNA levels of MHC class I heavy and light chains after normalization with the values for actin showed ~50–70% reduction compared with wild-type levels. The mRNA levels of MHC class I heavy chain and, to a lesser degree, light chain were also decreased in the thymi of IFNAR$^{-/-}$ and IFNGR$^{-/-}$ mice, showing that at least part of the regulation of basal class I expression requires an intact IFN signaling pathway. Similar reductions were also observed in splenocytes. To study the possible role of other transcription factors in reduced MHC class I expression, we examined the level of IRF-1 mRNA, which is a STAT1 target and has been shown to modulate the levels of both MHC class I heavy and light chain in response to IFN-γ (49, 50). IRF-1 mRNA levels were decreased substantially (30–50%) in spleens and thymi of STAT1$^{-/-}$ mice (Fig. 2 A) and to a lesser extent in lymphocytes from IFNAR$^{-/-}$ and IFNGR$^{-/-}$ mice. These results suggest that IFNs, operating through STAT1 and possibly IRF-1, are required to
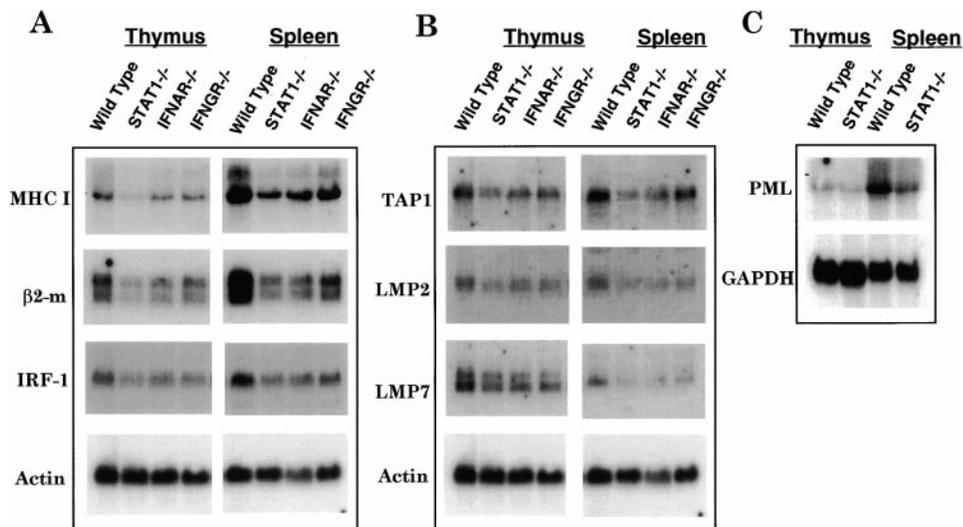
Figure 2. Genes for MHC class I antigen processing and presentation are regulated by STAT1. (A) Reduced expression of heavy and light chain mRNA in the absence of STAT1. 20 μg of total RNA prepared from lymphocytes of thymi or spleens of wild-type, STAT1$^{-/-}$, IFNAR$^{-/-}$, or IFNGR$^{-/-}$ mice were hybridized with ^{32}P-labeled probes specific for MHC class I heavy chain, light chain (β2m), and IRF-1. (B) Reduced constitutive mRNA for antigen processing and presentation–related molecules. Abundance of TAP1, LMP2, LMP7, and actin was measured as described for A. (C) PML is regulated by STAT1 and is differentially expressed in thymus and spleen. Abundance of PML and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was analyzed by Northern hybridization.

maintain full basal mRNA levels of MHC class I heavy and light chains. Interestingly, class I heavy and light chain mRNA expression was much higher in spleen than thymus (Fig. 2 A). Similarly increased cell surface class I protein levels were observed in peripheral relative to immature lymphocytes, suggesting a developmental upregulation of class I molecules during maturation.

As antigen processing and presentation molecules are also critical for surface expression of the MHC class I complex, we measured the mRNA levels of these accessory molecules. As shown in Fig. 2 B, TAP1, LMP2, and LMP7 expression was reduced to ∼35% of wild-type levels in splenocytes and thymocytes from STAT1$^{-/-}$ mice. Smaller reductions of TAP1, LMP2, and LMP7 mRNA levels were seen in thymus and spleen of IFNAR$^{-/-}$ and IFNGR$^{-/-}$ mice. These results showed that all of the MHC class I complex and related genes examined were regulated by STAT1 and that the regulation was at least partially due to constitutive IFN signaling. In all cases, loss of STAT1 produced a more severe reduction in MHC class I and accessory molecules than did loss of a single IFN receptor, suggesting that the phenotype of STAT1$^{-/-}$ mice was due to either the additive effect of loss of both type I and type II IFN receptors or to an IFN-independent mechanism.

A nuclear protein recently implicated in the control of MHC class I gene expression and antigen processing is the promyelocytic leukemia (PML) protooncogene (51). PML mRNA expression was reduced approximately twofold in spleen cells from STAT1$^{-/-}$ mice (Fig. 2 C). Because this protein may act as a master regulator of MHC class I cell surface expression that functions downstream of STAT1, its reduction in STAT1$^{-/-}$ cells may contribute to reduced MHC class I cell surface levels. Interestingly, like class I heavy and light chain genes, PML was also expressed at higher levels in spleen relative to thymus, indicating its potential involvement in the developmental switch in class I expression in lymphocytes.

STAT1 Regulates Constitutive MHC Class I Expression on Lymphocytes by a Cell-intrinsic Mechanism. Reduced expression of MHC class I molecules observed in the absence of STAT1 could be due to either a cell-intrinsic role for STAT1 in lymphocytes, e.g., downstream of a cytokine receptor, or to a more global role elsewhere in the animal, e.g., regulating production of a secreted cytokine that ultimately targets lymphocytes. To determine if the reduction of MHC class I was a primary, cell-autonomous defect, we performed bone marrow transplantation (BMT). Bone marrow cells from either wild-type or STAT1$^{-/-}$ mice were used to reconstitute either STAT1$^{+/+}$ or STAT$^{-/-}$ animals that had been lethally irradiated. To distinguish donor-derived lymphocytes from residual endogenous cells of the recipient mice, donor cells were infected with a retrovirus expressing GFP. The levels of MHC class I on mature peripheral blood lymphocytes were monitored 6 wk after BMT by staining with antibodies against MHC class I plus TCR for mature T cells or plus IgM for mature B cells and gating on GFP-positive lymphocytes.

Four types of transplants were performed: STAT1$^{+/+}$ cells transferred into STAT1$^{+/+}$ or STAT1$^{-/-}$ mice and STAT1$^{-/-}$ cells transferred into STAT1$^{+/+}$ or STAT1$^{-/-}$ mice. Wild-type and STAT1$^{-/-}$ bone marrow was capable of reconstituting both mature T and B lymphocytes in mice of both genotypes (Fig. 3). STAT1$^{-/-}$ bone marrow was somewhat less efficient at reconstituting mature T lymphocytes than wild-type donors, although the cause of this deficiency is unclear. Nonetheless, on both subsets of lymphocytes, MHC class I expression levels reflected the genotype of the donor rather than that of the recipient. STAT1$^{+/+}$ cells transferred into STAT1$^{-/-}$ hosts acquired an MHC class I phenotype similar to that of wild-type cells (Fig. 3, A and B, second rows from top), whereas STAT1$^{-/-}$ cells transferred to STAT1$^{+/+}$ hosts maintained their reduced levels of MHC class I (Fig. 3, third rows from top). Autologous transplants also maintained the level of MHC
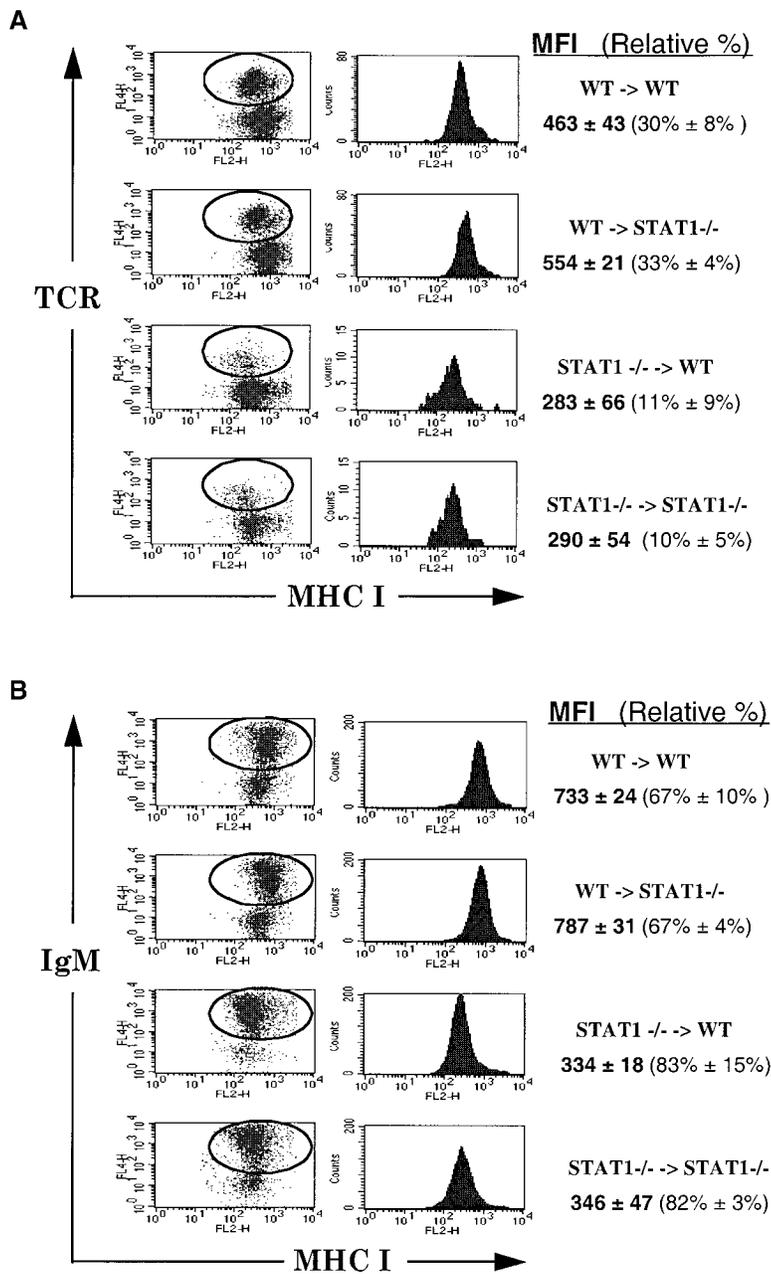
A

MFI   (Relative %)

WT -> WT
463 ± 43 (30% ± 8%)

WT -> STAT1-/-
554 ± 21 (33% ± 4%)

STAT1 -/- -> WT
283 ± 66 (11% ± 9%)

STAT1-/- -> STAT1-/-
290 ± 54 (10% ± 5%)

B

MFI   (Relative %)

WT -> WT
733 ± 24 (67% ± 10%)

WT -> STAT1-/-
787 ± 31 (67% ± 4%)

STAT1 -/- -> WT
334 ± 18 (83% ± 15%)

STAT1-/- -> STAT1-/-
346 ± 47 (82% ± 3%)

Figure 3. Expression of MHC class I is cell autonomous. Peripheral blood lymphocytes prepared after BMT were double stained with anti–H-2K^b–PE and anti–TCR-allophycocyanin (A) or anti–IgM-allophycocyanin (B), followed by FACS™ analysis gating on GFP-positive lymphocytes. Mean fluorescence intensity (MFI) is the average MHC class I intensity of GFP–TCR or GFP–IgM DP lymphocytes (circled regions) from three to four chimeric mice. The relative percentages of TCR$^+$ or IgM$^+$ cells among GFP$^+$ lymphocytes are shown.

class I appropriate for their genotype (Fig. 3, first and fourth rows from top). These results demonstrated that basal expression of MHC class I on T and B lymphocytes is regulated by a cell-intrinsic process that depends on the STAT1 status of the cell itself, independent of the environment in which it matures.

STAT1-dependent but IFN-independent Regulation of MHC Class I Expression in Mature T Lymphocytes. The greater reduction of class I heavy and light chain mRNA in STAT1$^{-/-}$ cells relative to IFN receptor mutants (Fig. 2) suggested that STAT1 may be important even in the absence of IFN. To further examine the role of constitutive IFN signaling in MHC expression patterns on different subsets of lymphocytes, we compared MHC class I antigen levels on

lymphocytes from mice devoid of IFN signaling by combined loss of receptors for both IFN-α and IFN-γ (Fig. 4). As noted above, higher levels of MHC class I expression were detected on lymphocytes from peripheral organs than on thymocytes. Different subsets of T lymphocytes also displayed different levels of class I. CD4$^+$CD8$^+$ DP thymocytes expressed very limited amounts of surface MHC class I compared with CD4$^-$CD8$^-$ DN or CD4$^-$CD8$^+$ and CD4$^+$CD8$^-$ SP thymocytes (Table I). In peripheral organs, the levels of MHC class I on both CD4 and CD8 T lymphocytes were further elevated compared with those of SP thymocytes.

In spite of the differential expression of MHC class I on different lymphocyte populations, when the levels of MHC
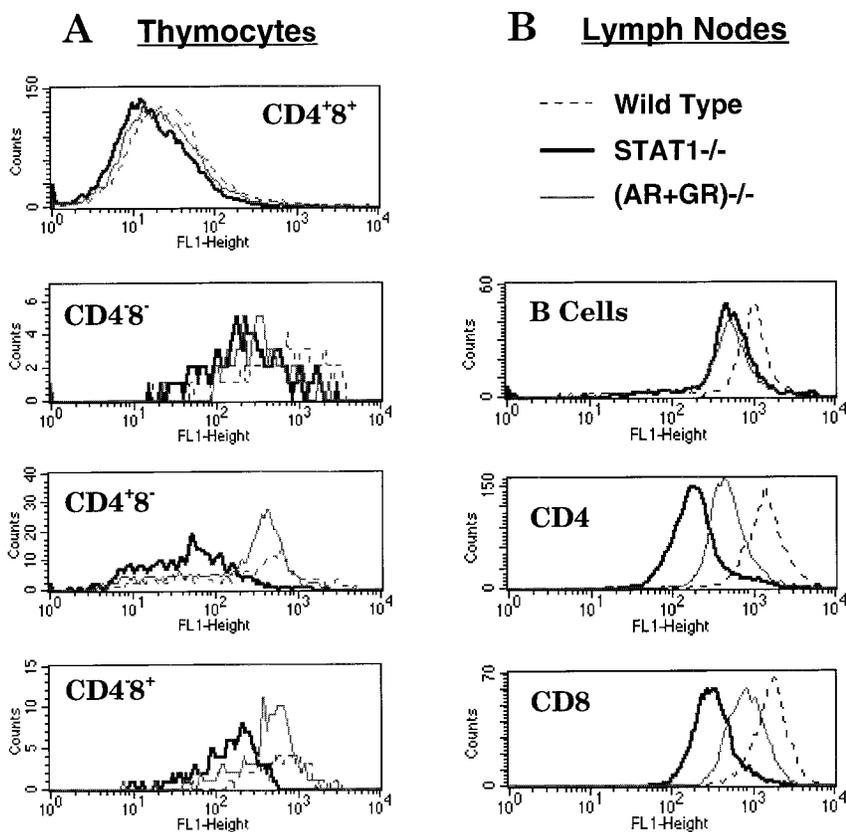
A Thymocytes

B Lymph Nodes

Figure 4. Reduced surface expression of MHC class I antigens in different subsets of STAT1$^{-/-}$ lymphocytes. Freshly isolated lymphocytes from thymi (A) or lymph nodes (B) of wild-type (dashed lines), STAT1$^{-/-}$ (bold lines), or (AR+GR)$^{-/-}$ (thin lines) mice were triple stained with CD4–TC, CD8–PE, and MHC class I–FITC, followed by FACS™ analysis. CD4$^-$CD8$^-$ lymphocytes from lymph nodes were regarded as B cells.

class I were compared between STAT1$^{-/-}$ and wild-type animals, all subsets of lymphocytes, whether from thymus, spleen, or lymph nodes, showed reduced expression of MHC class I in the absence of STAT1 (Table I and Fig. 4). Whereas DP and DN thymocytes and B lymphocytes from spleen and lymph nodes showed 40–50% reduction of MHC class I, SP thymocytes (CD4$^-$CD8$^+$ and CD4$^+$CD8$^-$) and peripheral T lymphocytes (both CD4 and CD8) showed a more pronounced reduction of MHC class I expression (only 16–26% of wild-type levels). MHC class I expression was also reduced on other cells types, including monocytes, macrophages, NK cells, and mouse embryonic fibroblasts (data not shown), suggesting that STAT1 is generally required for maintaining constitutive levels of MHC I expression.

RNA expression studies (Fig. 3) suggested that class I

levels regulated by STAT1 were at least partially independent of the presence of either IFN receptor. This observation left open the possibility that the more severe defect observed in the absence of STAT1 could be due to the combined loss of IFN-α and IFN-γ signaling. We therefore tested class I protein levels in the absence of both IFN-α and IFN-γ receptors using cells from (AR+GR)$^{-/-}$ mice. To exclude the possibility that any variation in MHC class I expression was due to differences in genetic background, wild-type, STAT1$^{-/-}$, IFNAR$^{-/-}$, and (AR+GR)$^{-/-}$ mice were maintained on the 129 (H-2^b) strain background.

A similar reduced level of MHC class I was observed on B cells of both spleens and lymph nodes from (AR+GR)$^{-/-}$ and STAT1$^{-/-}$ mice (Table I and Fig. 4 B). Therefore, the STAT1 requirement in B cells is likely to be entirely down-

Table I. *Mean Fluorescence Intensity of MHC Class I in Different Subsets of Lymphocytes*

	Thymocytes				Splenocytes			Lymph nodes		
	CD4$^-$8$^-$	CD4$^-$8$^+$	CD4$^+$8$^+$	CD4$^+$8$^-$	B	CD8	CD4	B	CD8	CD4
Wild type	974	706	69	531	974	1,562	1,368	1,097	1,709	1,520
STAT1$^{-/-}$	374	186	30	107	585	396	379	632	358	252
IFNAR$^{-/-}$	689	614	52	463	663	1,273	926	703	1,230	904
IFNGR$^{-/-}$	530	538	40	346	599	874	644	665	1,099	664
(AR+GR)$^{-/-}$	464	489	43	321	597	793	577	586	883	537

stream of IFN signaling. Surprisingly, however, SP thymocytes and CD4 and CD8 T lymphocytes from STAT1$^{-/-}$ mice displayed a more pronounced reduction of MHC class I (~50%) than those from (AR+GR)$^{-/-}$ mice. Therefore, STAT1 was required for the expression of MHC class I antigens on maturing and peripheral T lymphocytes even in the absence of any IFN signaling.

IFN-independent Phosphorylation and Nuclear Translocation of STAT1 in Resting T but not B Lymphocytes. The preceding results showed that endogenous IFNs were present and functional in normal animals but also indicated that STAT1-dependent gene expression could be independent of IFN signaling. These results prompted us to test the tyrosine phosphorylation and nuclear accumulation of STAT1, an indication of its activation by cytokines. Nuclear extracts prepared from freshly isolated splenocytes of wild-type, STAT1$^{-/-}$, and (AR+GR)$^{-/-}$ mice were subjected to immunoblot with antibody specific for tyrosine-phosphorylated STAT1. As shown in Fig. 5 A, top panel, phospho-STAT1α and phospho-STAT1β were indeed detected in nuclear extracts of wild-type resting splenocytes, presumably indicative of the action of IFN. Strikingly, a lower but still significant level of phospho-STAT1 was also detected in the (AR+GR)$^{-/-}$ splenocytes. Whereas the decreased activation of STAT1 in (AR+GR)$^{-/-}$ lymphocytes relative to wild-type cells was presumably due to the loss of IFN responsiveness, the residual phospho-STAT1 in (AR+GR)$^{-/-}$ lymphocytes must result from an IFN-independent mechanism. These results demonstrate that both IFN-dependent and -independent pathways contributed to the basal activation of STAT1 in lymphocytes.

MHC class I expression was differentially regulated in T and B cells; specifically, STAT1-mediated expression in B cells was entirely IFN dependent, yet both IFN-dependent and -independent pathways were involved in STAT1-mediated expression in T cells (Fig. 4). Therefore, we tested whether the regulation of basal activation of STAT1 also varied in these two subsets of lymphocytes. Nuclear extracts from isolated splenic T and B lymphocytes of wild-type and (AR+GR)$^{-/-}$ mice were analyzed for phospho-STAT1. As shown in Fig. 5 B, activated STAT1α and STAT1β were detected in both T and B lymphocytes of

wild-type mice. In contrast to wild-type lymphocytes, phospho-STAT1 levels were undetectable in B cells from (AR+GR)$^{-/-}$ mice but could still be detected in T cells. Therefore, STAT1 activity in B cells was entirely dependent on IFN signaling, whereas both IFN-dependent and -independent pathways mediated STAT1 activation selectively in T cells. This dual mode of STAT1 activation was not limited to splenic T lymphocytes; basal activation of STAT1 was also present in (AR+GR)$^{-/-}$ thymocytes (Fig. 5 B). This pattern of STAT1 activation mirrors the regulation we found for MHC class I gene expression.

Selective Activation of STAT1 by IL-7 in Mature T but not B Lymphocytes. The previous results defined an IFN-independent activation of STAT1 in T but not B cells. We reasoned that factors involved in selective activation of STAT1 might be cytokines preferentially acting on thymic and splenic T cells but not on mature B cells. One such candidate is IL-7, whose receptor is expressed on both T and B lineage progenitors (52–54) but not on DP thymocytes or mature peripheral B lymphocytes (55). STAT1 phosphorylation in freshly isolated splenocytes was blocked by the kinase inhibitor staurosporine (data not shown), suggestive of the action of a cytokine signaling pathway. Therefore, we first examined whether IL-7 could activate STAT1 in lymphocytes. STAT1 activation was significantly enhanced after either IL-7 or IFN-γ treatment of splenic T cells from wild-type mice (Fig. 6 A, top panel). A comparable level of activated STAT1 was present in T cells of (AR+GR)$^{-/-}$ mice after IL-7 treatment but not after IFN-γ treatment, due to the absence of IFN-γ receptors. STAT1 was also activated in thymocytes of wild-type and (AR+GR)$^{-/-}$ mice in response to IL-7 (data not shown). In marked contrast to T cells, no activation of STAT1 was observed in IL-7–treated B lymphocytes, although IFN-γ was still capable of activating STAT1 in B cells of wild-type though not (AR+GR)$^{-/-}$ mice (Fig. 6 A, bottom panel).

To further confirm the selective response to IL-7 in T versus B cells, electrophoretic mobility gel shift (EMSA) was performed using nuclear extracts from purified lymphocyte populations. Whereas IL-7 treatment induced the formation of two complexes in T cells from wild-type mice, IFN-γ only activated a complex with mobility correspond-
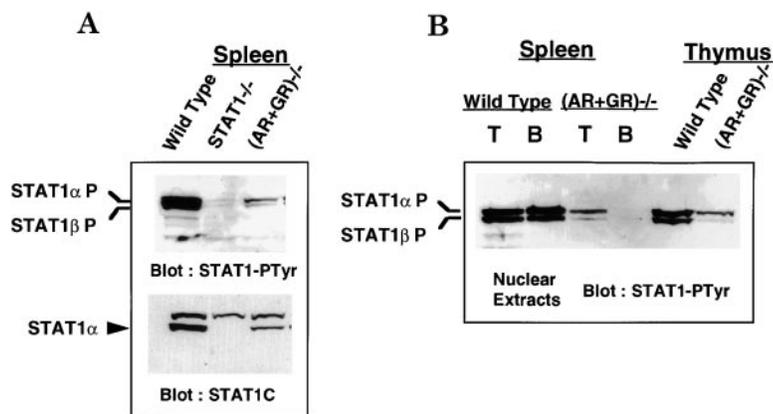
Figure 5. (A) Basal phosphorylation of STAT1 in splenocytes of wild-type and (AR+GR)$^{-/-}$ mice. Nuclear extracts prepared from 1.5×10^7 freshly isolated splenocytes of wild-type, STAT1$^{-/-}$, and (AR+GR)$^{-/-}$ mice were resolved in 7% SDS-PAGE and transferred to nitrocellulose membrane, followed by immunoblot with anti–phosphotyrosyl-STAT1 (STAT1-PTyr) antibody (top panel). The blot was then stripped and reblotted with anti-STAT1C antibody that only recognizes full length STAT1α (bottom panel). (B) Basal phosphorylation of STAT1 in B but not T cells completely depends on IFN signaling. Western blot as described for A was performed using nuclear extracts prepared from 10^7 splenic T or B cells or thymocytes of wild-type and (AR+GR)$^{-/-}$ mice.
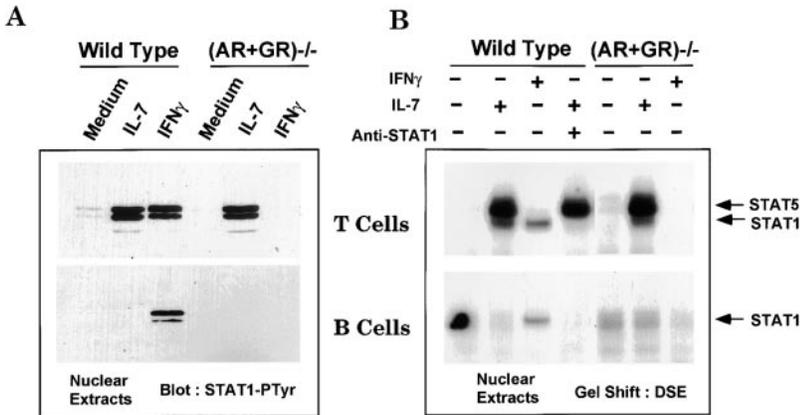
Figure 6. Differential activation of STAT1 by IL-7 in T and B cells. (A) Nuclear extracts prepared from selected T (top panel) or B (bottom panel) cells were left untreated or treated with IL-7 or IFN-γ for 30 min, followed by electrophoresis and immunoblot with anti–phosphotyrosyl-STAT1 (STAT1-PTyr) as described in the Fig. 5 legend. Basal phosphorylation of STAT1 was readily detected upon longer exposure (not shown). (B) EMSA was performed with nuclear extracts prepared as described for A in the presence or absence of antibody to STAT1C.

ing to the lower band of the IL-7–activated complexes (Fig. 6 B). Antibodies to STAT1 (Fig. 6 B, fourth lane from left) and STAT5 (not shown) identified these complexes as STAT1 (Fig. 6 B, bottom panel) and STAT5 (Fig. 6 B, top panel). However, whereas a STAT1 complex was activated by IFN-γ in B cells from wild-type mice, no complexes were activated in response to IL-7 in these cells (Fig. 6 B, bottom panel). IL-2 and IL-4, other cytokines capable of activating STAT5, failed to activate STAT1 (data not shown). Therefore, IL-7 selectively activated STAT1 in T but not B cells and functioned independently from IFN, consistent with the pattern previously observed for MHC class I expression.

IL-7–dependent Induction of MHC Class I Expression on T Cells. As STAT1 is essential for MHC class I expression, the differential activation of STAT1 in T and B cells by IL-7 prompted us to examine IL-7–dependent modulation of class I expression. Splenocytes and thymocytes of wild-type, $(AR+GR)^{-/-}$, and $STAT1^{-/-}$ mice were treated with IL-7 or IFN-γ for 48 h, and induction of class I protein expression was followed by FACS™ analysis (Table II). Whereas IFN-γ induced MHC class I expression on both T and B cells of wild-type mice, IL-7 selectively in-

duced class I expression only on T cells (Table II, top). This IL-7–dependent induction of MHC class I was not secondary to IFN signaling, as a comparable induction of MHC class I expression occurred on T cells from $(AR+GR)^{-/-}$ mice. IL-7 also induced class I on DN and SP thymocytes from wild-type mice but not on DP thymocytes that lack IL-7Rα (Table II, bottom). In contrast, neither IL-7 nor IFN-γ caused significant class I induction on cells deficient in STAT1, similar to the lack of effect of IL-7 on receptor-negative B cells. Analogous results were obtained with IL-7R–positive pre-B cell lines derived from bone marrow; MHC class I was induced in response to IL-7 on wild-type but not $STAT1^{-/-}$ cells (data not shown). Taken together, these results demonstrate that STAT1 is capable of mediating MHC class I induction in response to IL-7, and we conclude that STAT1 and IL-7 contribute to the basal level of MHC class I on mature T lymphocytes.

Discussion

We have demonstrated a role for constitutive IFN signaling in the absence of inflammation for homeostatic gene regulation in normal lymphocytes. This genetic and bio-

Table II. *Mean Channel Shift of MHC Class I Expression*

Splenocytes: Treatment	Wild type		$(AR+GR)^{-/-}$		$STAT1^{-/-}$	
	T	B	T	B	T	B
IFN-γ	76	379	0	0	6	0
IL-7	103	27	101	12	18	7

Thymocytes: Treatment	Wild type				$STAT1^{-/-}$			
	$CD4^+8^-$	$CD4^-8^+$	$CD4^-8^-$	$CD4^+8^+$	$CD4^+8^-$	$CD4^-8^+$	$CD4^-8^-$	$CD4^+8^+$
IFN-γ	97	52	69	60	1	0	1	1
IL-7	60	50	30	1	9	7	8	2

Splenocytes and thymocytes were treated with IFN-γ (100 U/ml) or IL-7 (10 ng/ml) for 48 h.

chemical analysis has also revealed underlying differences in the regulation of MHC class I expression in T and B cells. Moreover, these data uncovered a novel IFN-independent but IL-7–dependent STAT1 activity that operates selectively in T lymphocytes. A model for these different modes of regulation is illustrated in Fig. 7. In both T and B cells, constitutive IFN signaling activates STAT1 to maintain MHC class I gene expression either directly in combination with STAT2 and IRF-9 or possibly indirectly through its downstream target, IRF-1. In addition, both cell types have IFN-independent mechanisms for regulation of class I levels. In T cells, an IL-7–dependent but IFN-independent mechanism activates STAT1 phosphorylation, and this STAT1-dependent signaling is necessary for full class I expression. In B cells, the IFN-independent mechanism is also STAT1 independent, possibly acting through the B cell–specific protein CIITA that has been shown to modulate MHC class I gene expression (12, 13).

The role of IL-7 has been mainly characterized during lymphoid development, where it is required to prevent apoptosis in lymphoid precursors (34) and to serve as a cofactor for V(D)J recombination in both T and B lymphocytes (53, 54). These actions of IL-7 depend on JAK1, (56) JAK3 (57), and STAT5, though the requirement for STAT1 signaling is less clear (58, 59). Here we provide evidence that IL-7 is also important in mature T cells for maintenance of constitutive expression of MHC class I through activated STAT1. However, the role of STAT1 activation in the upregulation of MHC class I in response to IL-7 is likely indirect, possibly through IRF-1, a downstream target of STAT1. STAT1 can be activated in response to cytokines and growth factors other than IFN, at least in cell culture, but initial evidence from in vivo studies largely failed to support these alternative activators, suggesting instead that STAT1 was dedicated to IFN signaling (21, 36, 60). Recent evidence has suggested that fibroblast growth factor also functions through STAT1 in developing chondrocytes, at least in organ culture (28). IL-7–mediated STAT1 activation demonstrates a novel role for STAT1 outside the scope of IFN.

For B cells, no phospho-STAT1 accumulated in the absence of IFN signaling, and MHC class I levels were equivalent between STAT1$^{-/-}$ and (AR+GR)$^{-/-}$ mice. A STAT1-independent mechanism was operative for maintaining basal expression (Fig. 7), possibly through B cell–specific proteins such as CIITA, first characterized as regulators of MHC class II (12, 13). Indeed, overexpression of CIITA in human G3A cells, which are defective in CIITA, enhances the expression of MHC class I. It is likely that the action of CIITA compensates for the loss of IFN and STAT1 signaling in B cells derived from (AR+GR)$^{-/-}$ and STAT1$^{-/-}$ mice, allowing them to retain modest expression of MHC class I. The unchanged patterns of surface MHC class II (Fig. 1 B) and equal levels of CIITA mRNA in all mouse strains tested (data not shown) suggest that CIITA-dependent pathways are intact in the absence of STAT1, although their responsiveness to IFN is lost. Interestingly, expression of MHC class I on peripheral macrophages from STAT1$^{-/-}$ and (AR+GR)$^{-/-}$ mice were similar (data not shown). Macrophages also express CIITA and display high constitutive levels of MHC class II, consistent with a class I phenotype similar to that of B cells. CIITA is unable to perform this basal function in most tissues, as its expression outside the antigen presentation system is dependent on IFN induction. The important role of B lymphocytes and macrophages as APCs may explain why they have evolved independent mechanisms to maintain MHC expression.

We have demonstrated that basal activation of STAT1 in resting lymphocytes relies on the action of at least two different cytokines, namely IFN and probably IL-7. Although both IFN and IL-7 are involved in activating STAT1 in T lymphocytes, this action is entirely IFN dependent in B lymphocytes. Similarly, STAT1 basal phosphorylation is maintained by both IFN and IL-7 in SP and DN thymocytes but not in DP thymocytes that lack IL-7Rα and display very low levels of MHC class I. One of the functions of the constitutively activated STAT1 is to maintain MHC class I expression, although it is also likely that other targets exist as well. Interestingly, it has recently been shown that constitutively phosphorylated STAT1 accumulates in lung epithelium of asthmatic patients in the absence of IFN (61), suggesting that the tight regulation of STAT1 phosphorylation is important for normal physiology. Al-
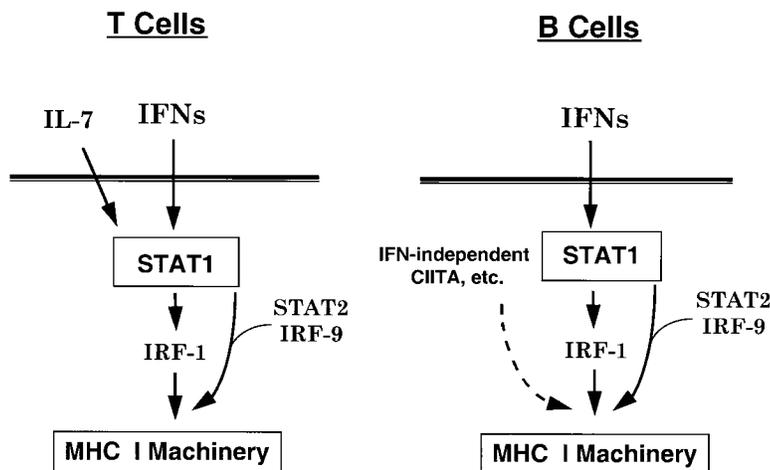
Figure 7. Differential regulation of constitutive MHC class I expression in T and B cells. Two pathways contribute to STAT1 activation that are essential for basal MHC class I expression in T cells. The first pathway is IFN dependent, whereas the second does not require IFN receptors and may depend on a cytokine, such as IL-7, to maintain MHC class I expression. In B cells, STAT1 activation is entirely dependent on IFN, whereas additional B cell–specific transcription factors, such as CIITA, contribute to the maintenance of MHC class I expression in a STAT1-independent manner.

though it has been known that IFN is responsible for increased class I expression during immune responses, we provide evidence for the continuous presence of IFNs in vivo in the absence of overt infection in the maintenance of basal levels of expression. The presence of endogenous IFNs in vivo has been suggested from low levels of IFN mRNA detected in various human organs (62), but the presence or significance of IFN protein in the absence of an inflammatory response has been less clear. Here we provide genetic and biochemical evidence for IFNs exerting a constitutive biological response. The source of basal IFN has not been determined, and we cannot exclude a role for subclinical infection or possibly normal intestinal flora. However, we suggest that basal IFN production, whether spontaneously produced or induced by common environmental stimuli, contributes to normal immune homeostasis. Consistent with this notion, gene-targeted loss of the inhibitor SOCS1 produced severe pathology (63) that appeared to be entirely due to unopposed IFN signaling (64a), again suggesting the presence of biologically active IFN in the absence of overt infection. Likewise, the transcriptional repressor IRF-2 appears to be essential to inhibit basal IFN responses, and ablation of IRF-2 leads to IFN-dependent pathology (64). These findings suggest a novel function for IFN as a homeostatic cytokine in addition to its role as an inflammatory mediator. Constitutive IFN signaling is required for beneficial responses, such as maintenance of MHC class I expression and perhaps priming a state of readiness for combating viral infection (65), whereas the less benign effects required only during inflammation are held in check by negative regulators, such as IRF-2 and SOCS1. It seems likely that other cytokines would have similar dual roles in both homeostatic and stress situations.

The mechanisms of developmental control of MHC class I expression are still largely uncharacterized. We show that expression of MHC class I is downregulated during the transition from DN to DP thymocytes, followed by reexpression on SP thymocytes. It was further enhanced when SP thymocytes matured to peripheral CD4 and CD8 T lymphocytes. Relative changes of MHC class I levels from DN to DP and from DP to SP thymocytes or SP thymocytes to peripheral T lymphocytes were comparable in wild-type and IFN receptor and STAT1 mutant mice (Table I), suggesting that neither IFN nor STAT1 is responsible for this developmental control. Interestingly, PML expression, a pos-

sible regulator of MHC class I downstream of STAT1 (51), was also higher in spleen than thymus; therefore, it may be one of the factors underlying developmental control.

IRF-1 has also been shown to affect MHC class I expression, inducing mRNA levels for heavy chain, LMP2, and TAP1 (66, 67). IRF-1 is a STAT1 target and was substantially reduced in STAT1$^{-/-}$ mice and, therefore, is likely one of the downstream mediators of STAT1 in this process. However, IRF-1 is not essential for regulation of MHC class I expression, at least in response to IFN-α/β, as MHC class I expression was still induced in IRF-1$^{-/-}$ cells, presumably through the action of the ISGF3 complex (24). Nonetheless, STAT1$^{-/-}$ and IRF-1$^{-/-}$ mice do not display identical phenotypes. Despite the decreased expression of MHC class I on STAT1$^{-/-}$ cells, we have not observed any abnormalities in CD8 development (data not shown), suggesting that the levels of MHC class I on thymic epithelium were still sufficient to rescue CD8$^+$ SP cells during positive selection. In contrast, IRF-1$^{-/-}$ mice show an increased ratio of CD4/CD8 cells due to an intrinsic requirement of IRF-1 for CD8 cell development (66).

One of the main functions of MHC class I is to facilitate CD8 T lymphocyte–mediated cell cytotoxicity during intracellular infection by viruses or bacteria. However, these pathogens have evolved many strategies to escape from immune responses by targeting the class I complex (68). For example, cytomegalovirus and adenovirus are able to retain MHC class I molecules in the endoplasmic reticulum or Golgi complex through a retrieval signal in the cytoplasmic tails of their viral proteins (69–71). HIV Nef protein modifies the endocytic machinery to downmodulate surface expression of MHC class I (72). Recently, JAK–STAT signaling was also shown to be disrupted during viral infection, resulting in suppression of MHC induction by IFN-γ (73, 74). The multiple modes of regulation of class I genes in B cells and other APCs may provide a backup system for the host during viral infection. When IFN signaling is targeted by viruses, the professional APCs would be less sensitive to the loss of IFN responsiveness in MHC class I regulation by relying on STAT1-independent pathways. Indeed, STAT1$^{-/-}$ mice displayed comparable virus clearance compared with wild-type mice after influenza virus infection (75). In vitro CTL activity against autologous targets after infection with influenza virus was also indistinguishable in STAT1$^{-/-}$ and wild-type mice (data not shown).

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