Cutting Edge: Class II Transactivator-Independent Endothelial Cell MHC Class II Gene Activation Induced by Lymphocyte Adhesion

Mark Collinge, Ruggero Pardi and Jeffrey R. Bender

*J Immunol* 1998; 161:1589-1593; ;
http://www.jimmunol.org/content/161/4/1589

**References**

This article cites 31 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/161/4/1589.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
NK cells induce MHC class II molecules on the surface of allogeneic endothelial cells in an adhesion-dependent, IFN-γ-independent manner. Here, we demonstrate that NK cells induce HLA-DR on the surface of a mutant cell line that is defective in IFN-γ-induced MHC class II expression. RNA analysis in these cells and in a cell line that is defective in class II trans-activator (CIITA) demonstrates that NK cell-induced HLA-DRα mRNA expression is also CIITA-independent. The Janus kinase-1-deficient cell line U4A expresses HLA-DRα mRNA in response to NK cell activation, and HLA-DRα promoter constructs transfected into these cells are induced by NK cells but not IFN-γ. These data indicate that the IFN-γ-independent component of the target cell HLA-DR expression induced by lymphocyte adhesion uses a signaling pathway that is distinct from the IFN-γ-dependent mechanism and also suggest that CIITA is not required. The Journal of Immunology, 1998, 161: 1589–1593.

Inducible expression of MHC class II (MHCII) molecules occurs primarily at the level of transcription, and a number of cis- and trans-activating factors have been described that are required for constitutive and inducible expression (reviewed in Ref. 1). Resting vascular endothelial cells (ECs) do not express MHCII molecules but can be stimulated to do so by IFN-γ and lymphocyte adhesion (2–4). IFN-γ induction of MHCII requires new protein synthesis, specifically of the class II transactivator (CIITA). Mutations of CIITA have been identified in all MHCII-deficient cell lines from bare lymphocyte syndrome complementation group A. CIITA expression itself is induced by IFN-γ (5), apparently via the Janus kinase (JAK)-1/STAT-1a pathway (6–8). Rather than binding to specific DNA sequences of the MHCII promoters, CIITA appears to interact directly with other transactivating factors (9–11).

NK cells are the most efficient cellular inducers of EC class II molecules (3). Although NK cells are competent producers of IFN-γ, NK-mediated HLA-DR induction at both the transcriptional and membrane level can be achieved in an IFN-γ-independent manner (12). Direct NK cell to EC contact is required, and the β2 integrin/ICAM-1 adhesion pathway is critical. Additionally, NK cells induce HLA-DRα mRNA more rapidly than does IFN-γ (12), indicating that new protein synthesis may not be required.

Here, we provide evidence that the HLA-DRα expression induced by NK cells can be achieved not only in an IFN-γ-independent manner, but also in the absence of CIITA expression. HLA-DR mRNA and protein were induced by NK cells in mutant cell lines that were defective in MHCII and CIITA expression. Additionally, HLA-DRα promoter constructs and mRNA were induced in a cell line that was deficient in JAK-1, which is a regulatory kinase that is essential for CIITA induction by IFN-γ. Taken together, these results indicate a pathway of HLA-DR induction by NK cells that is not only independent of the IFN-γR, but also of CIITA, which was previously believed to be essential for the induction of MHCII molecules.

Materials and Methods

Reagents

Anti-CD56 mAb was obtained from BioSource International (Camarillo, CA), rIFN-γ was supplied by Collaborative Biomedical Products (Bedford, MA), and neutralizing anti-IFN-γ was purchased from Genzyme (Cambridge, MA). Oligonucleotide primers were obtained from the W. M. Keck Biotechnology Resource Laboratory at Yale University (New Haven, CT). All other Abs and reagents used were as described previously (12).

Cell isolation and culture

NK cells were isolated from leukocyte-enriched products from single healthy adult donors; the products were obtained by leukopheresis that was performed at the Yale Pheresis Unit, as described previously (12). Immunodepletion (negative panning) led to NK cells that were >98% CD56−, CD16−, CD3−. Single-donor HUVECs were isolated and cultured as described previously (12) and used at less than passage 6. The HT-1080 cell line was purchased from Genzyme (Cambridge, MA). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and 2 mM L-glutamine. The cell lines HT-1080 and U4A were obtained from ATCC (Rockville, MD). The HT-1080 cell line expresses CIITA and is CIITA-dependent for HLA-DR induction. The U4A cell line is CIITA-deficient and CIITA-independent for HLA-DR induction. Both cell lines were grown in DMEM supplemented with 10% FCS and 2 mM L-glutamine.
line was obtained from American Type Culture Collection (Manassas, VA) (CCL-121) and maintained in Eagle’s MEM with nonessential amino acids and Earle’s balanced salt solution (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS. The 2C4, G2A, G3A, and U4A cell lines were obtained from George R. Stark (Cleveland Clinic Foundation, Cleveland, OH) and maintained in high glucose DMEM (Life Technologies) supplemented with 10% FBS.

Membrane HLA-DR induction assays and flow cytometry

Flow cytometric analysis was performed as described previously (12, 13).

NK cell panning

After recovering the NK/target cell coculture from plates with trypsinization, the panning of NK cells away from target cells was performed by immunodepletion with anti-CD16, -CD45, and -CD56 mAbs as described previously (12). RNA was purified from target cells that had been separated from NK cells and was subjected to RT-PCR (see below) with primers specific for CD45 to ensure complete NK cell depletion.

RNA analysis

Total cellular RNA was isolated using TRIzol reagent (Life Technologies) and the phase lock gel system (5 Prime–3 Prime, Boulder, CO). RT-PCR was performed using 1 µg of total RNA, Superscript II RTaseH reverse transcriptase (Life Technologies), and oligo(dT)12 primer (Promega, Madison, WI) according to the manufacturers’ instructions. PCR was performed as described previously (12) using the following primer pairs: ison, WI) according to the manufacturers’ instructions. PCR was performed by an overexpression of CIITA (K. Wright and J. Ting, DR that do not express surface MHCII molecules either at rest or in a dependent manner (12). To investigate the involved signaling pathway, we took advantage of a series of cell lines that had been rendered MHCII-deficient by chemical mutagenesis (15). The cell line was obtained from American Type Culture Collection (Manassas, VA) (CCL-121) and maintained in Eagle’s MEM with nonessential amino acids and Earle’s balanced salt solution (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS. The 2C4, G2A, G3A, and U4A cell lines were obtained from George R. Stark (Cleveland Clinic Foundation, Cleveland, OH) and maintained in high glucose DMEM (Life Technologies) supplemented with 10% FBS.

Membrane HLA-DR induction assays and flow cytometry

Flow cytometric analysis was performed as described previously (12, 13). The HLA-DRα promoter human growth hormone (HGH) constructs that were kindly provided by Richard Flavell (Yale University) have been used by us previously (12). The luciferase reporter pGL2 control vector (Promega) was cotransfected as a normalization control.

Transient transfections

Cells that had been plated to 50% confluence were transfected for 8 h with 10 µg of each plasmid using a calcium phosphate transfection protocol (14). After glycerol shock, cells were fed fresh medium and left overnight to recover. Fresh medium, medium containing 100 U/ml of IFN-γ, or allogeneic NK cells were added and incubated for 48 h. The HGH secreted into the medium was determined using a 125I-HGH radioimmunomassay kit (Nichols Institute, San Juan Capistrano, CA), and the HGH that normalized to luciferase activity in cell extracts was determined using a luciferase assay system (Promega) and a Lumat LB 9501 (Berthold, Wildbad, Germany) luminometer.

Results and Discussion

We demonstrated previously that allogeneic NK cells induce MHCII molecules on resting ECs in an IFN-γ-independent, adhesion-dependent manner (12). To investigate the involved signaling pathways, we took advantage of a series of cell lines that had been rendered MHCII-deficient by chemical mutagenesis (15). The cell line 2C4, which was derived from the human fibrosarcoma cell line HT-1080, was mutagenized with ICR-191; clones were selected that do not express surface MHCII molecules either at rest or in response to IFN-γ. One such cell line, G2A, shows partial HLA-DRα promoter occupancy, and MHCII expression can be complemented by an overexpression of CIITA (K. Wright and J. Ting, personal communication). We treated 2C4 and G2A with either 250 U/ml of IFN-γ or allogeneic NK cells (20:1 NK:responder ratio) for 72 h, followed by membrane HLA-DR analysis by flow cytometry. Figure 1 shows that both IFN-γ and NK cells induce the surface expression of HLA-DR in the parental cell line 2C4. As described earlier, IFN-γ failed to induce HLA-DR in the G2A cell line, but G2A retained NK cell responsiveness with a significant induction of membrane HLA-DR when cocultured with NK cells. This finding is consistent with previous observations of IFN-γ-independent, endothelial HLA-DR induction by NK cells and also provided a system whereby this phenomenon could be analyzed at the level of transcription.

In cells that do not constitutively express class II molecules, MHCII gene activation is required for induction. HLA-DRα gene transcription is not directly and immediately induced by IFN-γ, but does require new protein synthesis, specifically of CIITA (reviewed in Ref. 1). The kinetics of EC HLA-DRα mRNA induction by allogeneic NK cells are faster than those by IFN-γ (12), which led us to investigate whether newly synthesized CIITA is required for HLA-DRα induction by NK cells. We again took advantage of the class I-negative G2A cell line and of an additional cell line, G3A, that had been generated by the same means; G3A is CIITA and class II negative and is complemented to wild-type IFN-γ-mediated MHCII inducibility by expression of rCIITA (16). HUVECs and 2C4, G2A, and G3A cells were incubated for 8 h in the presence of either 100 U/ml of IFN-γ or NK cells (12:1 NK:responder ratio). For the NK cell treatments, the responder cells were pretreated with anti-IFN-γR Ab, and anti-IFN-γ-neutralizing Abs were maintained in the coculture. Following the treatments, NK cells were removed through Ab panning; RNA was isolated from the target cells and analyzed for CIITA and HLA-DRα mRNA expression by RPA (Fig. 2A). CIITA and HLA-DRα mRNA was absent in control resting cells, except for a trace amount of HLA-DRα in the cell line 2C4. As expected, IFN-γ significantly up-regulated HLA-DRα and CIITA mRNA in HUVECs and 2C4. In the G2A and G3A cell lines, there was a small but detectable level of HLA-DRα mRNA induction by IFN-γ; however, this level approximated the level of basal expression in the 2C4 cell line. A low level of CIITA mRNA was also detected in the cells that were treated with IFN-γ. All cell lines that
CIITA mRNA. This induction is not due to rapid CIITA mRNA turnover or to unusual kinetics of induction, as no significant CIITA transcript levels were observed over a wide time range (1–48 h) (data not shown). These results indicate that HLA-DRα mRNA can be induced in target cells by NK cells in a manner that is independent of CIITA. To fully demonstrate the absence of CIITA message in NK cell-induced target cells, the more sensitive but less quantitative RT-PCR method was used. The results are shown in Figure 2B and confirm the pattern of CIITA and HLA-DRα expression that was seen by RPA. No CIITA mRNA was detected by PCR in 2C4, G2A, or G3A that had been treated with NK cells. PCR using primers that were specific for CD45 was negative, confirming that NK mRNA was absent from the samples. The IFN-γ-independent component of the NK-mediated endothelial class II mRNA induction is variable, as described previously (12). Although HLA-DRα mRNA is induced by NK cells in the absence of CIITA in most experiments, CIITA mRNA is sometimes noted depending upon the NK cell and EC donors, since these experiments are conducted with single-donor cells for both allogeneic NK cells and ECs. Figure 2C demonstrates that the IFN-γ-neutralizing Abs used in the NK cell activation experiments completely inhibit EC CIITA and HLA-DRα mRNA induction by IFN-γ as determined by PCR. Adding dextran sulfate to ECs suppresses the induction of CIITA and MHCII gene expression by IFN-γ (17). We are able to reproduce these results and show that NK cells bind to vascular ECs in the presence of dextran sulfate; in addition, HLA-DRα mRNA is induced (data not shown).

All attempts to detect CIITA mRNA in the mutant cell lines that were cocultured with NK cells have failed, suggesting an alternative mechanism for MHCII gene activation. We also performed PCR with primers corresponding to nt 235–519 CIITA (18) to detect CIITA mRNA species that may contain 3′ truncations. The experimental results were identical with the PCR analysis using primers corresponding to nt 2881–3351 that is described above. CIITA mRNA can be induced from multiple promoters depending upon the specific cellular environment; at least four promoters have been described for human CIITA (19, 20) that lead to the expression of dextran sulfate; in addition, HLA-DRα mRNA is induced (data not shown).

Since it appears that the IFN-γ-independent induction of HLA-DRα by NK cells is also independent of CIITA, we investigated other molecules involved in IFN-γ signaling. JAK-1 and JAK-2 associate directly with the IFN-γR; upon phosphorylation, these kinases are able to phosphorylate and activate STAT-1 (reviewed in Ref. 21). STAT-1α then translocates to the nucleus and binds a γ-activation sequence (GAS) or GAS-like elements in the promoters of IFN-γ-activated primary response genes. At least one of the promoters for CIITA contains a GAS-like element (19, 20). Suppression of STAT-1α expression inhibits IFN-γ-stimulated CIITA mRNA expression (8). Since our coculture experiments used anti-IFN-γ and anti-IFN-γR Abs and since CIITA was not induced in target cells that were treated with NK cells, we investigated whether JAK-1 was required. The U4A cell line (22) is defective in IFN-γ signaling and expresses truncated JAK-1 protein (23). We used U4A in our coculture system to determine whether JAK-1 was required to obtain HLA-DRα induction by NK cells. IFN-γ (100 U/ml) or NK cells (5:1 NK:responder ratio) were added to confluent cultures of the U4A cell line or the parental cell line HT-1080 and incubated for 16 h. Following the removal of NK cells by Ab-mediated panning, target cell RNA was isolated and subjected to RT-PCR (Fig. 3A). No basal HLA-DRα message was detected in either cell line, but IFN-γ was able to induce both CIITA and HLA-DRα message in the parental cell line HT-1080;
subjected to PCR using primers specific for GAPDH, CIITA, HLA-DR, and total RNA was isolated from target cells, reverse transcribed, and (5:1 NK:responder ratio). NK cells were removed by Ab-mediated pan-

ever, NK cells induced HLA-DR

expression from the HT-1080 cell line, IFN-γ and NK cells (5:1 NK:responder ratio) induced the

gene expression, resulting in HLA-DRα gene activation.

According to DNA footprinting analysis, the HLA-DRα promoter in the G3A cell line (CIITA-defective) is completely bare; however, this promoter is partially occupied in the G2A cell line (K. Wright and J. Ting, personal communication). Efforts are under way to compare IFN-γ- and NK cell-induced footprints in these mutant cell lines as well as in ECs in an effort to identify distinct regions of the HLA-DRα promoter that is involved in gene activation by NK cells.

CIITA knockout mice express MHCII molecules in the inter-
digitating reticular cells of the thymus (27). An isotype-specific activator of HLA-DQ that can act independently of CIITA has been proposed (28, 29). There is precedent, therefore, for the endogenous expression of MHCII molecules in the absence of CIITA. Although we describe an IFN-γ-independent pathway, accessory pathways of MHCII induction by IFN-γ have been described previously (30) that involve non-Janus protein tyrosine kinases. Given the easily detectable HLA-DRα gene activation in the U4A cell line, it is possible that NK cell adhesion results in the activation of a similar, non-JAK-mediated kinase pathway. It is also worth noting that CIITA has a γ nt-binding motif that appears to be critical for its function (31). Other GTP-binding proteins may be induced in ECs by lymphocyte adhesion, by replacing CIITA in its protein-protein interaction role, or through other common roles of GTP-binding proteins, including signal transduction and protein transport. Here, we provide the first evidence that inducible MHCII (HLA-DRα) molecules can be expressed independently of CIITA. While it is likely that CIITA is required for IFN-γ induction of MHCII molecules (5), it can no longer be viewed as an essential regulator of MCHII in all cellular contexts.

Acknowledgments

We thank George Stark and Ian Kerr for providing the IFN-γ-signaling mutant cell lines and Kenneth Wright and Jenny Ting for helpful discussions. We also thank Lynn O’Donnell for expert technical assistance and both Louise Benson and Gwen Davis for cell culture support. We are grateful to the Milford General Hospital Delivery Room for providing umbilical cords and to the Yale Pheresis Unit and Rita Girdzis for assistance with leukopheresis.

References


