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Role of NK Cell-Activating Receptors and Their Ligands in the Lysis of Mononuclear Phagocytes Infected with an Intracellular Bacterium[1]

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We studied the role of NK cell-activating receptors and their ligands in the lysis of mononuclear phagocytes infected with the intracellular pathogen *Mycobacterium tuberculosis*. Expression of the activating receptors NKp30, NKp46, and NKG2D were enhanced on NK cells by exposure to *M. tuberculosis*-infected monocytes, whereas expression of DNAX accessory molecule-1 and 2B4 was not. Anti-NKG2D and anti-NKp46 inhibited NK cell lysis of *M. tuberculosis*-infected monocytes, but Abs to NKp30, DNAX accessory molecule-1, and 2B4 had no effect. Infection of monocytes up-regulated expression of the NKG2D ligand, UL-16 binding protein (ULBP)1, but not expression of ULBP2, ULBP3, or MHC class I-related chain A or chain B. Up-regulation of ULBP1 on infected monocytes was dependent on TLR2, and anti-ULBP1 abrogated NK cell lysis of infected monocytes. The dominant roles of NKp46, NKG2D, and ULBP1 were confirmed for NK cell lysis of *M. tuberculosis*-infected alveolar macrophages. We conclude that NKp46 and NKG2D are the principal receptors involved in lysis of *M. tuberculosis*-infected mononuclear phagocytes, and that ULBP1 on infected cells is the major ligand for NKG2D. Furthermore, TLR2 contributes to up-regulation of ULBP1 expression. ***The Journal of Immunology,*** **2005, 175: 4611–4617.**

Natural killer cells are a central component of the innate immune response, comprising the first line of defense against a variety of tumors and microbial pathogens, including viruses, bacteria, and fungal and other intracellular parasites (1–5). The lytic activity of NK cells is controlled by complex interactions of inhibitory and activating receptors with specialized signaling machinery (6–9). Some of the principal activating NK cell receptors are the natural cytotoxicity receptors, NKp30, NKp44, and NKp46, as well as NKG2D, 2B4 (CD244), and DNAX accessory molecule-1 (DNAM-1)[3] (CD226) (10, 11). The natural cytotoxicity receptors are exclusively expressed on NK cells. NKG2D and 2B4 are expressed on NK cells, $CD8^+$ cells, and γδ T cells (12–15), while DNAM-1 is expressed on NK and T cells (16).

NK cell-activating receptors have multiple ligands that trigger cytotoxicity. For example, NKG2D binds to the polymorphic MHC class I-related chain (MIC)A and MICB (12), which are expressed at low levels in gut epithelium and are up-regulated during heat shock or during infection with CMV (17, 18). NKG2D also binds to UL-16 binding proteins (ULBPs), which are MHC class I-like molecules that are expressed by tumor cell lines. ULBPs enhance the capacity of NK cells to lyse tumors and to produce cytokines such as IFN-γ and TNF-α (19, 20). For the natural cytotoxicity receptors, viral hemagglutinins were recently identified as ligands that bind NKp46 and NKp44 (21, 22), triggering lysis of infected cells. NKp30 and NKp46 also recognize membrane-associated heparan sulfate proteoglycans on tumor cells (23). The ligand for 2B4 (CD244) is CD48, and the ligands for DNAM-1 are poliovirus receptor (CD155) and Nectin-2 (CD112). These receptor-ligand interactions have been implicated in T cell and NK cell cytotoxicity (24–26).

Most studies of NK cell receptor-ligand interactions have evaluated lysis of tumor cells and virally infected cells, but little is known of the mechanisms that control lysis of mononuclear phagocytes that are infected with intracellular bacteria. We previously showed that NK cells used the NKp46 receptor to lyse *Mycobacterium tuberculosis*-infected monocytes (27). In the current study, we determined the relative contribution of five activating NK cell receptors to the lysis of infected monocytes and alveolar macrophages. In addition, we evaluated the ligands for NKG2D on *M. tuberculosis*-infected mononuclear phagocytes and studied the role of TLR2 in up-regulating expression of one of these ligands.

Materials and Methods

Patient population

Blood was obtained from 15 healthy donors. Bronchoalveolar lavage fluid was obtained from eight healthy donors. No donor had clinical or laboratory evidence of tuberculosis or HIV infection. In all donors for whom

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[3] Abbreviations used in this paper: DNAM, DNAX accessory molecule; ULBP, UL-16 binding protein; MIC, MHC class I-related chain; MOI, multiplicity of infection.

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bronchoscopy was performed, the pathologic process was unilateral, and bronchoalveolar lavage was performed in the normal lung. Informed consent was obtained from all donors. All studies were approved by the Institutional Review Board of the University of Texas Health Center (Tyler, TX).

Cytofluorometric analysis and Abs

The following Abs were used for immunolabeling: FITC anti-CD14, FITC anti-CD3, PE-conjugated anti-CD56, FITC anti-2B4, and PE-conjugated anti-DNAM-1 (all from BD Pharmingen), and immunostaining was performed by standard techniques (28). In some cases, indirect immunolabeling was performed using mouse mAb to human NKG2D (M585), MICA/B (6D4, as ascites), ULBP1, ULBP2, and ULBP3 (M295, M312, and M551, respectively; Amgen), and mouse polyclonal antisera specific for NKp46 and NKp30 as primary Abs (21) and a FITC goat anti-mouse secondary Ab (Southern Biotechnology Associates).

Based on forward and side light scatter characteristics, we gated on lymphocytes or monocytes, and the percentage of cells labeled with specific surface markers was determined with a FACSCalibur (BD Biosciences).

The mAbs to NKG2D, MICA/B, ULBP1, ULBP2, and ULBP3, and antisera to NKp46 and NKp30 used for immunolabeling were also used for neutralization experiments. The DNAM-1 mAb DX11 and 2B4 mAb 2-69 (both from BD Pharmingen) were used for neutralization.

Reagents for studies of TLR2

We used an Ab against TLR2 (2392), which was a kind gift of Dr. P. Godowski (Genentech, San Francisco, CA) and a hexameric peptide Pam_3-Cys-Ser-Ser-Asn-Lys-Ser-OH of the 19 kD *M. tuberculosis* lipoprotein (29), which was generously donated by Dr. R. Modlin, University of California (Los Angeles, CA).

Culture of alveolar macrophages

Bronchoalveolar lavage fluid was passed through sterile gauze and centrifuged at 834 × *g* for 5 min. The cell pellet was resuspended in RPMI 1640, and 1.2×10^6 cells were allowed to adhere to each well of flat-bottom 12-well plates. Approximately 90% of the bronchoalveolar lavage cells were macrophages, as judged by Giemsa staining. Nonadherent cells were removed, and adherent cells were >98% alveolar macrophages. Some of the adherent cells were uninfected and others were infected with single cell suspensions of *M. tuberculosis* strain H37Ra at a multiplicity of infection (MOI) of 20:1.

Preparation of target cells for cytotoxicity assays

PBMC were isolated by differential centrifugation over Ficoll-Paque (Amersham Pharmacia Biotech). Monocytes were isolated with magnetic beads conjugated to anti-CD14 (Miltenyi Biotec), and positively selected cells were >95% $CD14^+$, as measured by flow cytometry. Monocytes (10^6/well) were plated in 12-well plates (BD Labware) in 1 ml of RPMI 1640 (Invitrogen Life Technologies) containing 10% heat-inactivated human serum (Pel-Freez Biologicals). Some monocytes were uninfected and others were infected with single cell suspensions of *M. tuberculosis* strain H37Ra at a MOI of 5:1, as previously described (30). Cells were incubated for 4 h at 37°C in a humidified 5% CO_2 atmosphere, washed to remove extracellular bacilli, and cultured in RPMI 1640 containing 10% heat-inactivated human serum. Approximately 25–40% of the monocytes were infected. After 48 h, cells were collected using a cell scraper, and used as targets in cytotoxicity experiments. These cells were >90% monocytes, as assessed by staining with nonspecific esterase. Cell viability was >90%, as judged by trypan blue exclusion.

In some experiments immature dendritic cells, prepared by treating monocytes with 20 U/ml of GM-CSF and 10 U/ml of IL-4 for 4 days, were used as targets. In other experiments Chinese hamster ovary (CHO)-K cells were used as targets.

Isolation of NK cells

$CD3^+$ cells were depleted from PBMC with magnetic beads conjugated to anti-CD3 (Miltenyi Biotec), and from the negative cell fraction, $CD56^+$ cells were isolated by positive selection with magnetic beads conjugated to anti-CD56 (Miltenyi Biotec). The positive cells were 95–100% $CD56^+$ and 95–97% $CD3^-$, as measured by flow cytometry, and were used as effector cells.

Culture of monocytes and NK cells

Infected and uninfected monocytes or alveolar macrophages were plated in round-bottom 96-well plates (Costar) at 5×10^3/well in 200 μl of RPMI 1640 containing penicillin (Invitrogen Life Technologies) and 10% heat-inactivated human serum in the presence or absence of NK cells (2×10^5/well at a 40:1 E:T cell ratio).

Cytotoxicity assay

NK cell-mediated cytotoxicity against infected and uninfected monocytes and alveolar macrophages was assayed in a ^{51}Cr release assay, using standard methods (31). Briefly, target cells were labeled overnight with 100 μCi of sodium chromate. Target cells were washed three times, and triplicate wells of 10^4 cells/well were mixed with effector cells at an E:T ratio of 40:1 in 200 μl of RPMI 1640 with 10% heat-inactivated human serum. Ten hours after incubation, 100 μl of supernatant was removed from each well, and radioactivity was measured in a gamma counter. The percentage of lysis was calculated using: 100 × [(experimental release − spontaneous release/maximum release − spontaneous release)].

Statistical analysis

Results are shown as the mean ± SE. For data that were normally distributed, comparisons between groups were performed by a paired or unpaired *t* test, as appropriate. For data that were not normally distributed, the Wilcoxon rank-sum test was used. Values of $p < 0.05$ were considered statistically significant.

Results

Surface expression of NK cell-activating receptors

To determine the surface distribution of NK cell activating receptors after exposure to *M. tuberculosis*-infected monocytes, we isolated NK cells and monocytes from PBMC of healthy donors. NK cells were cultured with uninfected or *M. tuberculosis*-infected monocytes, as outlined in *Materials and Methods*, and stained with Abs to the NK cell-activating receptors. Expression of NKp46 was markedly increased on NK cells upon exposure to *M. tuberculosis*-infected cells, and expression of NKp30 and NKG2D were also up-regulated (Fig. 1). In contrast, expression of DNAM-1 and 2B4 was not up-regulated on NK cells after coculture with infected monocytes (data not shown).

NK cell lysis of M. tuberculosis*-infected monocytes is mediated through NKp46 and NKG2D*

To determine the relative contribution of the different receptors to lysis of infected monocytes, we incubated freshly isolated NK cells from five healthy donors with mAb or antisera to the five NK cell receptors, before addition of *M. tuberculosis*-infected monocytes. Anti-NKp46 and anti-NKG2D reduced the net percentage of lysis of infected monocytes from 29 ± 8% to 10 ± 3% ($p = 0.01$) and

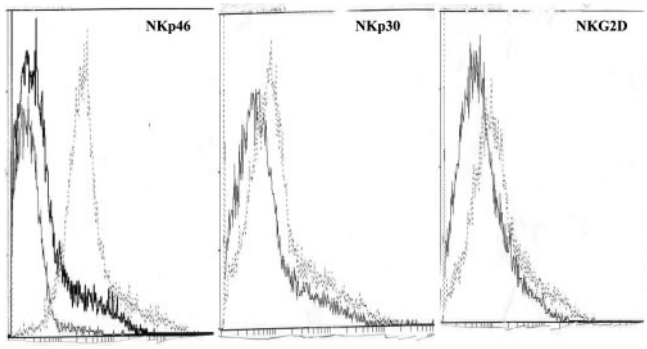

FIGURE 1. Expression of the NK cell receptors NKp46, NKp30, and NKG2D after exposure to uninfected and *M. tuberculosis*-infected monocytes. Staining with Abs to NKp46, NKp30, and NKG2D of NK cells, cultured for 10 h with infected monocytes (dotted line) or uninfected monocytes (thick solid line). The thin solid line shows staining of NK cells with mouse IgG. Cells from four donors showed similar results, and one representative experiment is shown.

to 7 ± 5% ($p = 0.01$), respectively (Fig. 2*A*). In contrast, neutralization of NKp30, DNAM-1, and 2B4 did not reduce lysis of infected monocytes (Fig. 2*B*). Anti-NKp30 inhibited NK cell lysis of autologous immature dendritic cells from five donors (6 ± 3% vs 17 ± 5%; $p = 0.01$) as previously described (32), indicating that this Ab neutralized NKp30. Similarly, anti DNAM-1 reduced NK cell lysis of CHO-K cells from 24 ± 2% to 14 ± 2% ($p = 0.01$) as previously described (24). Anti-NKG2D and anti-NKp46 both inhibited the capacity of NK cells from six donors to lyse *M. tuberculosis*-infected monocytes in a dose-dependent manner (data not shown). We also determined the synergic effect of anti-NKG2D and anti-NKp46 on the lysis of infected monocytes. At suboptimal concentration (5 μg/ml) anti-NKG2D and anti-NKp46 Abs totally inhibited the lysis (Fig. 2*C*).

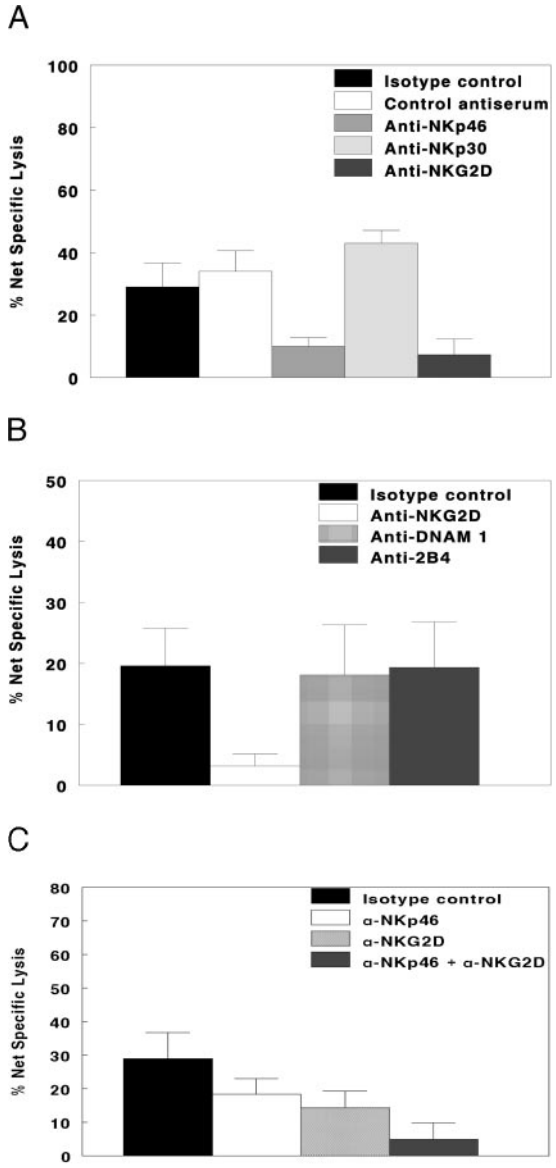

FIGURE 2. Effect of neutralization of NK cell receptors on the capacity of NK cells to lyse *M. tuberculosis*–infected monocytes. *A*, Neutralization of NKp46, NKp30, and NKG2D. NK cells from five healthy donors were preincubated with either Ab or antiserum for 1 h on ice, washed, and cultured with autologous infected monocytes in the presence of the same Ab or antiserum. The Abs used were anti-NKG2D or an isotype control Ab, both at 10 μg/ml. Antisera to NKp46, NKp30, or control antiserum, all at 10 μl/ml were used. Mean values with SEs for percentage net specific lysis by NK cells are shown. *B*, Neutralization of NKG2D, DNAM-1, and 2B4. NK cells from five healthy donors were cultured with autologous infected monocytes in the presence of mAbs to NKG2D, 2B4, or DNAM-1 or of isotype control Ab (all at 10 μg/ml). Mean values with SE for the percentage of net specific lysis by NK cells are shown. *C*, Synergistic effect of anti-NKp46 and anti-NKG2D Abs. NK cells from five healthy donors were cultured with autologous infected monocytes in the presence of mAbs to NKp46 (5 μg/ml) and NKG2D (5 μg/ml). Mean value with SE for the percentage of net specific lysis by NK cells is shown.

ULBP1 is the major NKG2D ligand expressed by M. tuberculosis*-infected monocytes*

The data above suggest that NKp46 and NKG2D are the dominant receptors that mediate lysis of *M. tuberculosis*-infected monocytes. NKG2D recognizes a variety of ligands, such as MICA, MICB, and the proteins ULBP1, ULBP2, and ULBP3, which are normally expressed at low levels but are up-regulated during stress (19). To identify the ligands for NKG2D on *M. tuberculosis*-infected monocytes, we measured cell surface ligand expression on infected and uninfected $CD14^+$ monocytes from five healthy donors by flow cytometry. ULBP1 expression was significantly up-regulated on infected monocytes, compared with uninfected cells (12.5 ± 2.4% vs 5.2 ± 1.3%; $p = 0.002$) (Fig. 3). In contrast, there were no differences in expression of MICA/B, ULBP2, and ULBP3 on infected and uninfected monocytes (Fig. 3).

Effect of anti-ULBP1 on NK cell-mediated lysis

To confirm that ULBP1 was the major ligand responsible for NK cell-mediated lysis of infected monocytes, we cultured freshly isolated NK cells with *M. tuberculosis*-infected monocytes, in the presence of anti-ULBP1 or isotype control Abs (10 μg/ml). Anti-ULBP1 significantly inhibited NK cell-mediated lysis of infected monocytes (net specific lysis 0 vs 29 ± 8%, $p < 0.001$) (Fig. 4).

NK cells lyse alveolar macrophages through NKp46 and NKG2D

The most physiologically relevant mononuclear phagocytes involved in defense against *M. tuberculosis* are alveolar macrophages, which *M. tuberculosis* first encounters in the lungs. To determine whether these current findings with monocytes apply to alveolar macrophages, we tested the capacity of NK cells from four donors to lyse *M. tuberculosis*-infected allogeneic alveolar macrophages. We also performed similar experiments with NK cells and autologous alveolar macrophages from three additional donors. NK cells lysed *M. tuberculosis*-infected alveolar macrophages to a markedly greater extent than uninfected macrophages

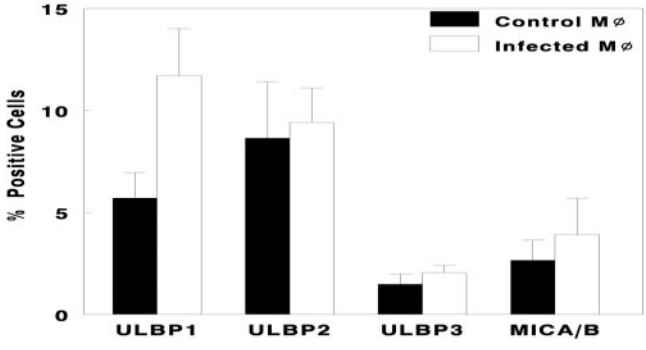

FIGURE 3. Expression of NKG2D ligands by infected and uninfected monocytes (Mϕ). Monocytes from five healthy donors were infected with *M. tuberculosis* strain H37Ra. After 48 h, $CD14^+$ cells were stained with Abs to different NKG2D ligands, and the percentage of positively stained cells was determined. Mean value with SE is shown.

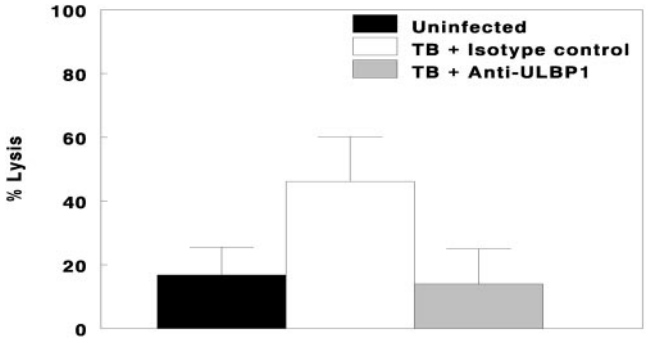

FIGURE 4. Effect of anti-ULBP1 on the capacity of NK cells to lyse *M. tuberculosis*-infected monocytes. Freshly isolated NK cells from five healthy donors were cultured with uninfected monocytes, or with *M. tuberculosis*-infected monocytes (TB) in the presence of 10 μg/ml anti-ULBP1 or isotype control Ab. Mean value with SE is shown.

(mean net specific lysis of 38 ± 8% for allogeneic macrophages and 30 ± 2% for autologous macrophages) (Fig. 5).

To determine whether NKp46- and NKG2D-mediated NK cell lysis of *M. tuberculosis*-infected alveolar macrophages, we incubated NK cells from five donors with *M. tuberculosis*-infected alveolar macrophages in the presence of Abs to different NK cell receptors. Anti-NKp46 and anti-NKG2D reduced net specific lysis of infected alveolar macrophages to 1 ± 1% and 2 ± 1%, respectively ($p < 0.001$ for both), compared with net specific lysis of 25 ± 9% with control antisera (Fig. 6). Anti-NKp30 did not inhibit lysis of infected alveolar macrophages, paralleling results obtained with monocytes shown in Fig. 2*A*.

ULBP1 is the major receptor for NKG2D in infected alveolar macrophages

To determine whether ULBP1 was the dominant receptor for NKG2D in infected alveolar macrophages, we incubated NK cells with infected autologous alveolar macrophages, in the presence of Abs to different NKG2D ligands. Anti-ULBP1 significantly inhibited lysis (14 ± 11% vs 46 ± 14%, $p < 0.001$), but anti-MICA/B did not (Fig. 7). The sum of these findings suggests that NKp46 and NKG2D are the major NK cell-activating receptors that control lysis of *M. tuberculosis*-infected alveolar macrophages, and that ULBP1 is the major NKG2D ligand used.

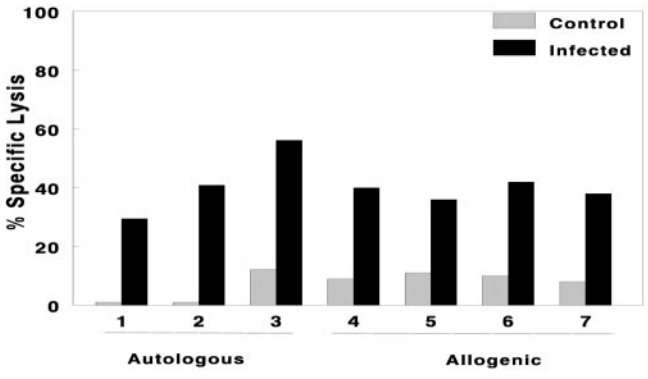

FIGURE 5. Cytotoxicity of NK cells against uninfected (control) and H37Ra-infected alveolar macrophages. NK cells were incubated with autologous or allogeneic alveolar macrophages, which were either uninfected or infected with H37Ra. Percentage of specific lysis of control and infected monocytes is shown.

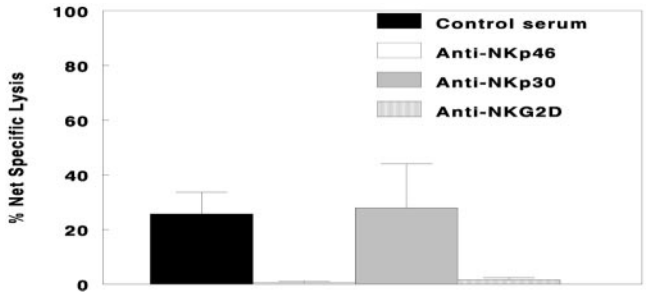

FIGURE 6. Effects of anti-NKp46, anti-NKG2D, and anti-NKp30 on NK cell-mediated lysis of alveolar macrophages. Freshly isolated NK cells from five healthy donors were cultured with infected and uninfected autologous alveolar macrophages in the presence of anti-NKG2D (10 μg/ml), anti-NKp46, anti-NKp30, or control antisera (all at 10 μl/ml), and net specific lysis was determined. Mean value with SE is shown.

TLR2-mediated up-regulation of ULBP1 expression

TLR signaling through the MyD88 adaptor up-regulates transcription of the retinoic acid inducible-1 family of NKG2D ligands in mice (33). Because the *M. tuberculosis* 19-kD lipoprotein binds to TLR2 and activates macrophages to produce monokines and Ag presentation (34), we investigated the possibility that TLR signaling in *M. tuberculosis*-infected mononuclear phagocytes induces expression of ULBP1. Monocytes from five healthy donors were cultured in medium alone, infected with H37Ra at an MOI of 5:1, or cultured with the hexameric peptide of the 19-kD *M. tuberculosis* lipoprotein. After 48 h, monocytes were stained with anti-ULBP1. The percentage of ULBP1-expressing cells increased from 2.0 ± 0.4% in uninfected cells to 7.4 ± 1.3% in *M. tuberculosis*-infected cells and to 7.7 ± 2.1% in monocytes stimulated with the lipopeptide (Fig. 8). When *M. tuberculosis*-infected cells were incubated with anti-TLR2, ULBP1 expression was inhibited (3.2 ± 1.1% vs 7.4 ± 1.3%, $p < 0.001$) (Fig. 8*A*). Anti-TLR2 inhibited NK cell lysis of monocytes stimulated with the hexameric peptide (1.4 ± 1% vs 15.8 ± 3.4%, $p < 0.03$) (Fig. 8*B*). This suggests that TLR2 mediates up-regulation of ULBP1 expression on infected monocytes.

Discussion

NK cells play a central role in the innate immune response to viral infection, and many elegant studies have delineated the receptor-ligand interactions and intracellular signaling pathways that mediate NK cell lysis of virally infected targets. In contrast, little is

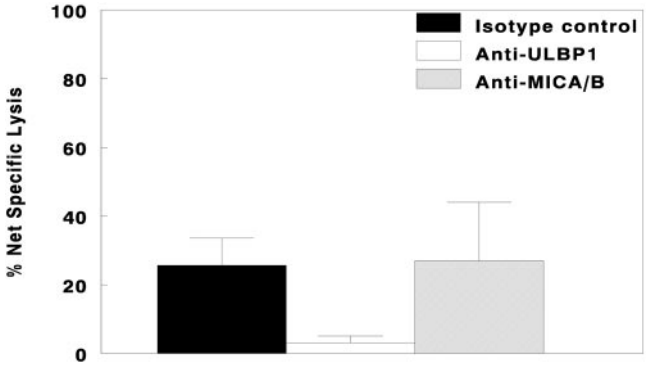

FIGURE 7. Effect of Abs to NKG2D ligands on NK cell-mediated lysis of alveolar macrophages. Freshly isolated NK cells from five healthy donors were cultured with *M. tuberculosis*-infected and uninfected autologous alveolar macrophages, in the presence of 10 μg/ml anti-ULBP1 or isotype control Ab, or a 1/100 dilution of anti-MICA/B. Net specific lysis was determined, and mean value with SE is shown.

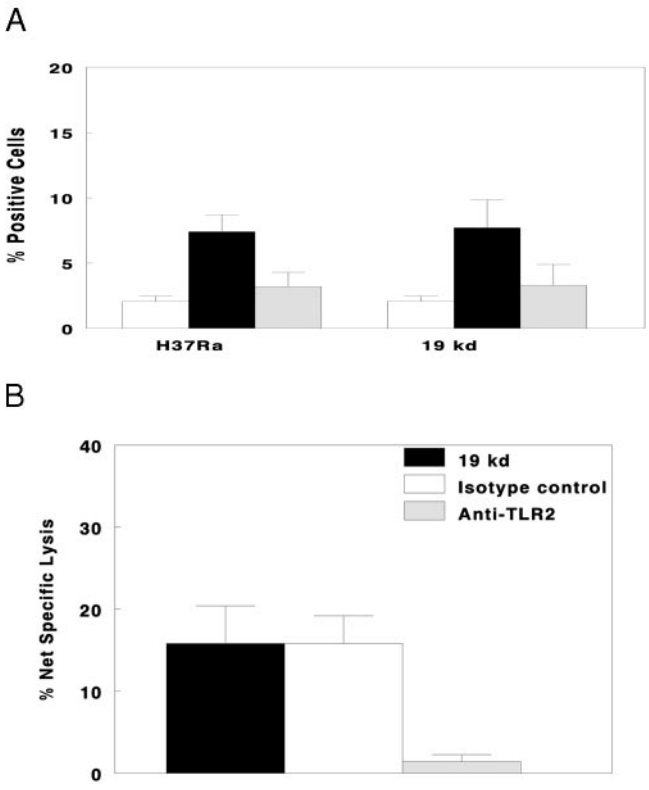

FIGURE 8. Effect of neutralization of TLR2. *A*, TLR2-mediated up-regulation of ULBP1 expression. Monocytes from five healthy persons were incubated alone (□) or with either a TLR2 stimulus (H37Ra or the hemaxeric peptide of the 19-kD *M. tuberculosis* lipoprotein (0.25 μg/ml)) and an isotype control Ab (10 μg/ml) (■) or a TLR2 stimulus and anti-TLR2 (10 μg/ml) (▨). Monocytes were incubated with the Abs on ice for 1 h, before addition of H37Ra or the hexameric peptide. After 48 h, cells were stained with anti-ULBP1. Mean value with SE is shown. *B*, Neutralization of TLR2 on the capacity of NK cells to lyse *M. tuberculosis*–infected monocytes. Monocytes from five healthy persons were cultured with the hexameric peptide of the 19-kD *M. tuberculosis* lipoprotein, as described in *A*. After 48 h, cells were used as target cells for autologous NK cells. Net specific lysis was determined, and mean value with SE is shown.

known about the mechanisms by which NK cells contribute to defense against intracellular bacteria. In this study, we demonstrated that NKp46 and NKG2D play important roles in the lysis of *M. tuberculosis*-infected monocytes. Of five NKG2D ligands, only ULBP1 was up-regulated in infected monocytes, and anti-ULBP1 inhibited NK cell lysis of infected monocytes, suggesting that lysis was mediated through interactions between NKG2D and ULBP1. Up-regulation of ULBP-1 and NK cell lytic activity depended on expression of TLR2, suggesting that *M. tuberculosis*-induced signaling through the TLR pathway can contribute to NK cell activity. The dominant roles of NKp46, NKG2D, and ULBP1 in NK cell-mediated lysis of infected monocytes were confirmed by studies of *M. tuberculosis*-infected alveolar macrophages, which are one of the first cells to encounter intracellular pulmonary pathogens. As far as we are aware, these represent the first studies of interactions between human NK cells and alveolar macrophages. Our results indicate that NK cells may play an important role in the initial stages of the local immune response to *M. tuberculosis* infection.

NKp46, NKp30, and NKp44 are exclusively expressed on NK cells and play a major role in NK cell-mediated cytotoxicity. NKp46 and NKp30 are constitutively expressed, but NKp44 is present only on activated NK cells. Because NKp44 mRNA is not up-regulated in NK cells exposed to *M. tuberculosis*-infected monocytes (27), we focused our current study on NKp30 and NKp46. Infected monocytes expressed higher levels of NKp46 than NKp30, and only neutralization of NKp46 inhibited NK cell lysis of infected mononuclear phagocytes, indicating that NKp30 did not play a significant role in this process. Because TGF-β down-regulates surface expression of NKp30 but not NKp46 (35), and *M. tuberculosis*-infected monocytes produce a high concentration of TGF-β (36), which may inhibit expression of NKp30, limiting its role in NK cell lysis of these cells.

Although mammalian ligands for NKp46 and NKp30 have not been identified, it is likely that such ligands are present on specific cell types. For example, dendritic cells are thought to express ligands for NKp30 but not NKp46 (32). In contrast, our results suggest that infected monocytes and alveolar macrophages express ligands for NKp46 but not NKp30. The NKp46 ligand on *M. tuberculosis*-infected macrophages may be structurally similar to viral hemagglutinin, which binds to NKp46 but not NKp30 (21, 22).

2B4 is an activating receptor on NK cells that binds to CD48 on target cells and triggers cytotoxicity by a subpopulation of NK cells (26). Although exposure of NK cells to *M. tuberculosis*-infected monocytes up-regulated expression of 2B4, this receptor did not play a primary role in lysis of infected cells, suggesting that abnormalities in 2B4 expression or signaling should not enhance susceptibility to tuberculosis. This finding is consistent with clinical evidence that patients with X-linked lymphoproliferative disorder, who have defective signaling through 2B4 but normal NKp46 function (37), are highly susceptible to EBV infection but not to infections with intracellular bacteria (10).

DNAM-1 is a transmembrane glycoprotein of the Ig superfamily that is constitutively expressed by NK cells, T cells, and monocytes (38, 39). When DNAM-1 on NK cells binds to the poliovirus receptor (CD155) or to Nectin-2 (CD112), this can result in lysis of certain tumor targets (24). These DNAM-1 ligands are highly expressed on endothelial cells, but not on monocytes (40). We found that DNAM-1 did not play a primary role in the capacity of NK cells to lyse *M. tuberculosis*-infected monocytes, suggesting that DNAM-1 ligands are not markedly up-regulated on infected mononuclear phagocytes. Alternatively, in polyclonal NK cell populations for which cytotoxicity is controlled by several NK receptors, the effects of NKp46 and NKG2D and their ligands may dominate over effects of DNAM-1 and its ligands, similar to findings reported for cytolytic activity of primary NK cells against tumor targets (24).

NKG2D interacts with six ligands: MICA and MICB, ULBP1, ULBP2, ULBP3, and ULBP4. MICA and MICB are not expressed by most cell types, but are induced in epithelial cells during heat shock (24) and in endothelial cells and fibroblasts that are infected with CMV (41). MICA was up-regulated in monocyte-derived dendritic cells after infection with extremely large numbers of *M. tuberculosis* (MOI of 2000:1) (42). In contrast, we found no increase in MICA/B expression during infection with smaller numbers of organisms (MOI of 5:1), which is likely to be more representative of conditions due to natural infection. The four ULBPs are differentially regulated by specific stimuli. For example, fibroblasts infected with CMV express ULBP1, ULBP2, and ULBP3 at different time points after infection (43). Our results indicate that infection of mononuclear phagocytes with *M. tuberculosis* up-regulates ULBP1, but not the other NKG2D ligands.

TLRs are central to innate immune responses by mononuclear phagocytes, and ligands for TLR2, TLR3, TLR5, and TLR9 directly stimulate human NK cells to produce IFN-γ and to lyse tumor cell targets (44, 45). In contrast, the TLR9 ligand, CpG

oligodeoxynucleotide activates NK cells through soluble factors produced by plasmacytoid dendritic cells (46). We found that a hexameric peptide of the 19-kDa lipoprotein of *M. tuberculosis*, a ligand for TLR-2 (34), up-regulates expression of the NKG2D ligand ULBP1 on *M. tuberculosis*-infected monocytes, providing a third potential mechanism by which TLRs modulate the function of NK cells. Enhanced ULBP1 expression is likely to stimulate binding to NKG2D, facilitating NK cell lysis of infected cells. Our findings extend recent observations in a murine model, in which TLR2, TLR3, and TLR4 up-regulated expression of the NKG2D ligands, retinoic acid early inducible-1 proteins (33), which share 20% homology with ULBP1 (47).

In summary, we found that NKp46 and NKG2D are the dominant receptors that contribute to NK cell lysis of *M. tuberculosis*-infected mononuclear phagocytes, including alveolar macrophages. The major NKG2D ligand on infected mononuclear phagocytes is ULBP1, and its expression depends on TLR2. Our results indicate that interactions between the TLR2 pathway and NK cells can contribute to the local immune response to *M. tuberculosis* infection.

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Disclosures

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