The CD16$^-$/CD56^{bright} Subset of NK Cells Is Resistant to Oxidant-Induced Cell Death[1]

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Phagocyte-derived reactive oxygen species ("oxygen radicals") have been ascribed a suppressive role in immunoregulation by inducing dysfunction and apoptotic cell death in lymphocytes. Earlier studies show that human NK cells are exceptionally sensitive to oxygen radical-induced apoptosis and functional inhibition. Two subsets of human CD56$^+$ NK cells have been identified: the highly cytotoxic CD56^{dim} cells which constitute >90% of NK cells in peripheral blood, and the less cytotoxic but efficiently cytokine-producing CD56^{bright} cells. In this study, we demonstrate that the CD56^{bright} subset of NK cells, in contrast to CD56^{dim} cells, remains viable and functionally intact after exposure to phagocyte-derived or exogenously added oxygen radicals. The resistance of CD56^{bright} cells to oxidative stress was accompanied by a high capacity of neutralizing exogenous hydrogen peroxide, and by a high cell-surface expression of antioxidative thiols. Our results imply that CD56^{bright} NK cells are endowed with an efficient antioxidative defense system that protects them from oxygen radical-induced inactivation. *The Journal of Immunology,* **2007, 179: 781–785.**

Natural killer cells are large granular lymphocytes that comprise up to 15% of the lymphocyte population in human peripheral blood (1). As innate effectors, NK cells are considered to play a critical role in the early phase of the immune response to pathogens and malignant cells before an adaptive immune response has developed (2, 3). Upon encounter with a tumor cell or a virus-infected cell, NK cells can become activated and kill the target cell by releasing preformed granules containing perforin and granzymes. When activated, NK cells modulate the adaptive immune responses by secreting cytokines, primarily IFN-γ (4, 5). Approximately 90–95% of human NK cells in peripheral blood display a relatively low density of the CD56 Ag on the cell surface (CD56^{dim}) and also express the low-affinity IgG FcR, FcγRIII (CD16). The CD56^{dim} NK cells produce low amounts of cytokines but express high levels of perforin and granzymes and thus exert high natural and Ab-dependent cellular cytotoxicity against susceptible target cells (6).

A smaller subset of peripheral blood NK cells (5–10%) expresses the CD56 Ag at a higher density and also typically lacks the CD16 Ag (CD56^{bright}16$^{dim/neg}$ phenotype). CD56^{bright} NK cells differ from CD56^{dim} cells by more efficiently producing cytokines and by exerting lower natural cytotoxicity (7, 8). Rather than forming part of the cytotoxic armaments of innate immunity, the CD56^{bright} cells appear to play a role at the innate/adaptive immunity interface, as reflected by their expression of CD62L and CCR7, which are adhesion molecules needed for homing to secondary lymphoid organs (9, 10). CD56^{bright} cells are thus localized in T cell areas of human lymph nodes where they interact with homing dendritic cells (11–13). By releasing IFN-γ, the CD56^{bright} NK cells are assumed to shape the adaptive immune response, promoting Th1 polarization (14).

Oxidative stress, defined as cellular toxicity inflicted by reactive oxygen species ("oxygen radicals"), has been ascribed a role in immunomodulation. First, oxygen radicals have been proposed to contribute to the dysfunction of cytotoxic lymphocytes characteristic of malignant disorders and chronic infections (15–20). Second, oxygen radicals reportedly suppress autoimmunity and arthritis development by controlling the cell-surface level of antioxidative thiols on lymphocytes (21, 22). Although these functional consequences of oxygen radical-related immunoregulation have been studied in some detail, relatively little is known about the sensitivity of individual lymphocyte subsets to oxidative stress. Earlier studies show that NK cells, in contrast to, e.g., CD4$^+$ T cells, are rapidly inactivated and highly prone to acquire features of apoptosis upon exposure to oxygen radicals, delivered either as exogenous hydrogen peroxide or produced by adjacent phagocytic cells (23–26). In this study, we report that CD56^{bright} NK cells are unexpectedly resistant to oxidative stress, based on the finding that CD56^{bright} cells, in contrast to CD56^{dim} cells, remain viable and functional after exposure to oxygen radicals. Our results imply that CD56^{bright} NK cells are endowed with a strong antioxidative defense system that protects them from oxidant-induced inactivation.

Materials and Methods

Separation of leukocytes

Peripheral venous blood was obtained as freshly prepared acid citrate dextrose-containing leukopacks from healthy blood donors at the Blood Centre (Sahlgren's University Hospital, Göteborg, Sweden). To remove catalase-containing erythrocytes, the blood (65 ml/donor) was mixed with 92.5 ml of Iscove's DMEM, 35 ml of 6% dextran, and 7.5 ml of acid citrate dextrose. After incubation for 15 min at room temperature, the supernatant was carefully layered on top of a Ficoll-Hypaque (Lymphoprep) density gradient. After centrifugation at 380 $\times$ *g* for 15 min, mononuclear cells were collected at the interface, while the pellet contained erythrocytes and polymorphonuclear phagocytes (PMN)[3] (25). Mononuclear cells were further

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[3] Abbreviations used in this paper: PMN, polymorphonuclear phagocyte; MP, mononuclear phagocyte; MFI, median fluorescence intensity; ALM-633, Alexa-633 C5-maleimide; PARP, poly(ADP-ribose)polymerase; AIF, apoptosis-inducing factor.

separated into lymphocytes and mononuclear phagocytes (MP) using a countercurrent centrifugal elutriation technique in which the sedimentation rate of cells in a spinning rotor is balanced by a counterdirected flow through the chamber. To this end, the mononuclear cells were resuspended in elutriation buffer, i.e., buffered NaCl supplemented with 0.5% BSA and 0.1% EDTA, and fed into a Beckman J2-21 ultracentrifuge with a JE-6B rotor (Beckman Coulter) at 2100 rpm. By slowly increasing the flow rate through the chamber, fractions of cells of well-defined sizes were collected. A fraction with >90% mononuclear phagocytes was obtained at a flow rate of ~19 ml/min. The procedure was repeated for the pellet recovered after the Ficoll-Hypaque gradient centrifugation, and a fraction with >95% PMN (with >98% neutrophilic granulocytes) was obtained at flow rates of >22 ml/min. Lymphocyte fractions recovered at flow rates between 14 and 16 ml/min were enriched for NK cells and T cells and contained <3% phagocytes. These lymphocyte fractions were pooled and subjected to further separation.

The elutriated lymphocytes were depleted of CD3$^+$ cells (NK cell-enriched lymphocytes) using IMag CD3 magnetic particles (BD Biosciences) according to the instructions provided by the manufacturer. In some experiments, the NK cell-enriched lymphocytes were stained with anti-human mAbs to CD3, CD16, CD56, and CD8, and >98% pure populations of CD3$^-$/16$^+$/56^{dim} and CD3$^-$/16$^-$/56^{bright} were sorted out using a BD FACSAria equipped with three laser lines (405, 488, and 633 nm) and FACSDiva version 5 software (BD Biosciences). Separated cells were resuspended in Iscove's DMEM supplemented with 10% human AB$^+$ serum and this medium was used in all experiments below unless otherwise stated.

Lymphocyte cell death

Lymphocytes, enriched NK cells, or FACS-sorted NK cells were incubated overnight in 96-well round-bottom plates with medium, autologous mononuclear phagocytes, hydrogen peroxide (H_2O_2), or autologous PMN. After 18 h, end-stage oxidant-induced cell death in NK cells was assayed using flow cytometry, based on the altered light-scattering characteristics displayed by end-stage apoptotic cells, i.e., a reduced forward scatter and an increased right angle scatter (23). Apoptosis was confirmed using annexin V and To-Pro-3 staining as described elsewhere (26). Apoptotic CD56^{dim} NK cells characteristically lose the expression of CD16 and CD56 (27). Therefore, in mixed lymphocyte preparations, the percentage of apoptotic CD56^{dim} cells was determined by comparing the fraction of live CD56^{dim} cells after exposure to oxidants with the fraction in the untreated control: percentage of apoptotic cells = 100 × (percentage of live CD56^{dim} of total lymphocytes in control − percentage of live CD56^{dim} of total lymphocytes in experiment)/(percentage of live CD56^{dim} of total lymphocytes in control). To investigate PJ34-mediated protection of NK cells, the H_2O_2 concentration that induced at least 30% apoptosis in the CD56^{bright} subset in the absence of PJ34 was used for analysis. That concentrations varied between experiments (range: 125–250 μM). The presented data indicate the fraction of dead NK cells in the control that were protected by addition of PJ34.

Intracellular staining for IFN-γ

NK cell-enriched lymphocytes were incubated overnight with autologous phagocytes MP/NK ratio 0.5 and 1) or hydrogen peroxide. After 18 h, cells were stimulated with 100 ng/ml PMA and 1 ng/ml ionomycin as described previously (28). After 1 h, brefeldin A (GolgiPlug; BD Biosciences) was added to retain IFN-γ intracellularly. After another 4 h, cells were harvested, washed, and stained for cell-surface Ags. Lymphocytes were then fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) and stained for intracellular IFN-γ using a PE-conjugated mAb.

Staining for cell-surface thiols

The relative number of cell-surface thiols on lymphocytes was determined using Alexa-633 C5-maleimide (ALM-633; Invitrogen Life Technologies) (22, 29). Freshly isolated PBMCs were incubated with 5 μM ALM-633 for 15 min on ice. After extensive washing, cells were stained with Abs directed against appropriate surface Ags and data were acquired and analyzed using a BD FACSCanto II with FACSDiva version 5 software. Results are presented as median fluorescence intensity (MFI) values of ALM-633 staining.

Hydrogen peroxide consumption assay

FACS-sorted preparations of CD56^{dim} and CD56^{bright} NK cell subsets were resuspended in Kreb's Ringer glucose buffer (500,000 cells/ml) and incubated with 30 μM hydrogen peroxide for 30 min at 37°C. The remaining hydrogen peroxide was detected using an isoluminol-ECL technique (30). Isoluminol (10 μg/ml) and HRP (4 U/ml) were added and the chemiluminescence activity was monitored for 30 s using a Mithras LB 940 plate reader (Berthold Technologies).

Abs and reagents

The following anti-human mAbs were purchased from BD Biosciences: anti-CD3 (PerCP, allophycocyanin), anti-CD8 (AmCyan, PerCP, allophycocyanin), anti-CD16 (FITC, allophycocyanin-Cy7), anti-CD56 (PE, PE-Cy7, allophycocyanin), anti-CD107a (PE-Cy5). Anti-CD3 (Pacific blue) and anti-IFN-γ (PE) were obtained from Invitrogen Life Technologies. FITC- and PE-conjugated annexin V were from BD Biosciences. The following compounds were used: *N*-ethyl maleimide, isoluminol, PJ34 (Sigma-Aldrich); dextran (Kabi Pharmacia); acid citrate dextrose (Baxter); BSA (ICN Biomedicals); EDTA and hydrogen peroxide (VWR); Ficoll-Hypaque, Lymphoprep (Nycomed); To-Pro-3, ALM-633 (Invitrogen Life Technologies); HRP (Boehringer Mannheim).

Statistics

Paired samples *t* tests were used throughout the study. For MFI values, data were transformed logarithmically before statistical calculation. All reported *p* values are two-sided.

Results

The CD56^{bright} NK cell subset is resistant to oxidant-induced cell death

We investigated the susceptibility of CD56^{dim} and CD56^{bright} cells to the toxicity of reactive oxygen species, derived from autologous phagocytes or added exogenously as hydrogen peroxide. NK cell-enriched lymphocytes were incubated with hydrogen peroxide, MP, or PMN overnight and assayed for end-stage apoptosis. CD56^{dim} NK cells were highly sensitive to exogenously added hydrogen peroxide and to phagocyte-derived oxygen radicals. In contrast, CD56^{bright} cells were almost completely resistant to oxidant-induced apoptosis when incubated with phagocytes or at lower H_2O_2 concentrations. At higher concentrations of hydrogen peroxide, CD56^{bright} cells were significantly less prone to acquire features of apoptosis than CD56^{dim} cells (Fig. 1). The difference in viability between CD56^{bright} and CD56^{dim} NK cells after exposure to oxidants also remained after 72 h of culture, indicating that the observed difference was not due to delayed cell death in the CD56^{bright} subset (data not shown).

To ensure that the observed resistance of CD56^{bright} cells to oxidants was not mediated by selective loss of CD16 and up-regulation of CD56 in surviving CD56^{dim} cells, pure preparations of CD56^{dim} and CD56^{bright} NK cells were recovered by cell sorting and then exposed to oxidants. As shown in Fig. 2, sorted CD56^{dim} cells were significantly more sensitive to oxidants than CD56^{bright} cells.

Apoptosis in CD56^{bright} NK cells is poly(ADP-ribose)polymerase (PARP) dependent

We have recently shown that oxidant-induced cell death in NK cells is mediated by the PARP/apoptosis-inducing factor (AIF) axis. Thus, exposure of NK cells to oxidants activates nuclear PARP, which in turn triggers translocation of mitochondrial AIF to the nucleus with ensuing DNA fragmentation and cell death (26). To investigate the role of the PARP/AIF axis for the oxidant-induced cell death in CD56^{bright} NK cells, we pretreated NK cells with a PARP inhibitor, PJ34, before exposing them to hydrogen peroxide. As shown in Fig. 3, the pronounced degree of apoptosis in CD56^{dim} cells as well as the lower degree of apoptosis in CD56^{bright} cells were efficiently prevented by PJ34, thus suggesting that the mechanisms of oxidant-induced cell death is similar in these NK cell subsets.

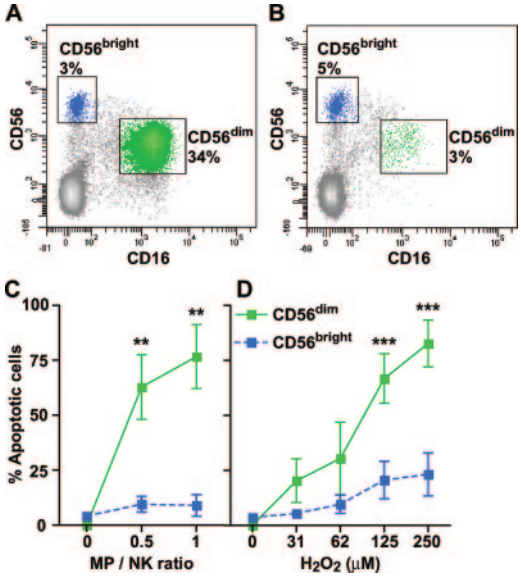

FIGURE 1. CD56^{bright} NK cells are resistant to oxidant-induced cell death. NK cell-enriched lymphocytes were incubated overnight in the absence (*A*) or presence (*B*) of mononuclear phagocytes at a 1:1 ratio. Dot plots show viable CD3$^-$ lymphocytes based on a live scatter gate. In *B*, a selective loss of live CD56^{dim} NK cells is observed, while the population of CD56^{bright} NK cells remains intact, as indicated by the increased fraction of CD56^{bright} cells among viable lymphocytes after exposure to phagocytes. Similar results were observed in six different experiments. In *C* and *D*, NK cell-enriched lymphocytes were exposed to mononuclear phagocytes at indicated ratios or concentrations of hydrogen peroxide overnight and assayed for end-stage apoptosis. CD56^{dim} cells were significantly more sensitive to phagocyte-derived radicals as well as to hydrogen peroxide than CD56^{bright} cells. Data are mean ± SEM. (MP/NK ratio 0.5, $p < 0.01$, $n = 6$; MP/NK ratio 1, $p < 0.01$, $n = 6$; 125 μM H_2O_2, $p < 0.001$, $n = 8$; 250 μM H_2O_2, $p < 0.001$, $n = 4$; **, $p < 0.01$; ***, $p < 0.001$).

CD56^{bright} NK cells remain functional after exposure to oxygen radicals

Next, we sought to determine whether CD56^{bright} cells remained functionally intact after exposure to phagocytes or hydrogen peroxide. For this purpose, we investigated the capacity of NK cell subsets to express intracellular IFN-γ after activation. After incubation with oxidants, NK cell-enriched lymphocytes were stimulated with PMA and ionomycin and stained for intracellular IFN-γ. In line with the previous findings that CD56^{bright} cells are the principal cytokine-producing NK cells (7), a significantly higher

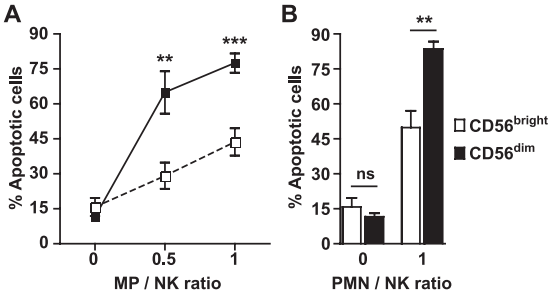

FIGURE 2. Phagocytes induce cell death in CD56^{dim} cells. FACS-sorted CD56^{bright} and CD56^{dim} cells were incubated overnight with mononuclear (*A*) or polymorphonuclear (*B*) phagocytes at indicated phagocyte to lymphocyte ratios. CD56^{bright} cells were significantly more resistant to phagocyte-induced cell death than CD56^{dim} cells (MP/NK ratio 0.5, $p < 0.01$, $n = 8$; ratio 1, $p < 0.0001$, $n = 12$; PMN/NK ratio 1, $p < 0.01$, $n = 5$; **, $p < 0.01$; ***, $p < 0.001$).

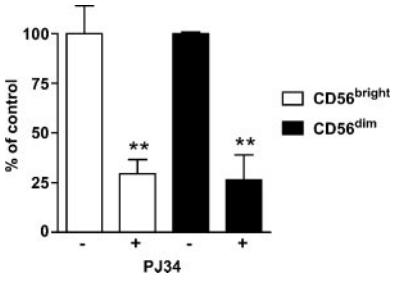

FIGURE 3. A PARP-1 inhibitor, PJ34, protects both NK cell subsets from oxidant-induced cell death. NK-enriched lymphocytes were preincubated with 0.5 μM PJ34 before exposure to hydrogen peroxide. Data are from the H_2O_2 concentration that induced at least 30% apoptosis in the CD56^{bright} subset (range: 125–250 μM). Data are normalized and displayed as a percentage of the corresponding control without PJ34. Thus, for both NK cell subsets, PJ34 protected 70–75% of the otherwise apoptosing NK cells (CD56^{bright}, $p < 0.01$, CD56^{dim}, $p < 0.01$, $n = 4$; **, $p < 0.01$).

percentage of CD56^{bright} cells stained positively for IFN-γ and these cells were to a significantly larger extent IFN-γ^{bright} as compared with CD56^{dim} cells. After addition of hydrogen peroxide or incubation with mononuclear phagocytes, the responsiveness to PMA/ionomycin stimulation dropped in CD56^{dim} cells. In contrast, CD56^{bright} cells preserved their capacity to produce IFN-γ after exposure to oxygen radicals (Fig. 4). In line with results from earlier studies in T cells, higher concentrations of hydrogen peroxide were required to induce NK cell death than those required to suppress NK cell function (31).

CD56^{bright} cells express more cell-surface thiols and neutralize hydrogen peroxide more efficiently than CD56^{dim} cells

Cells are endowed with elaborate systems for protection against the detrimental effect of oxidants. Central in these antioxidative

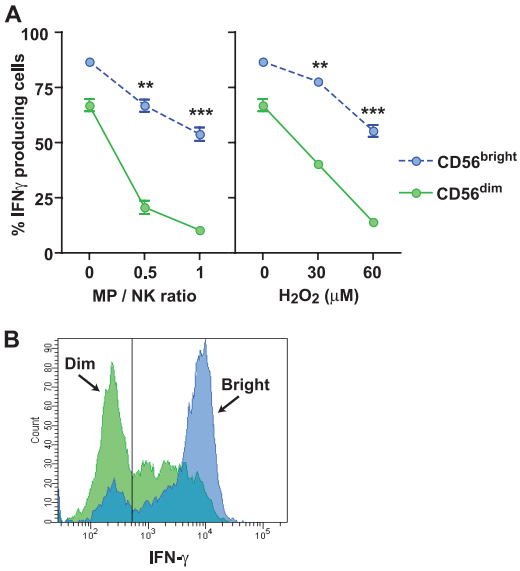

FIGURE 4. CD56^{bright} NK cells remain responsive to activation after exposure to oxidants. NK-enriched lymphocytes were exposed to mononuclear phagocytes or hydrogen peroxide overnight, and assayed for intracellular IFN-γ after stimulation with PMA/ionomycin. *A*, CD56^{dim} cells lost their ability to produce IFN-γ after exposure to radicals, while a significantly higher fraction of CD56^{bright} cells remained functional (MP/NK ratio 0.5, $p<0.01$; ratio 1, $p < 0.0001$; H_2O_2 30 μM, $p < 0.01$; 60 μM, $p < 0.0001$, $n = 7$). In *B*, a representative experiment with PMA/ionomycin-stimulated NK cells shows that CD56^{bright} cells expressed more IFN-γ on a per cell basis and were thus stained more intensely (higher MFI) than CD56^{dim} cells ($p = 0.01$, $n = 6$; **, $p < 0.01$; ***, $p < 0.001$).

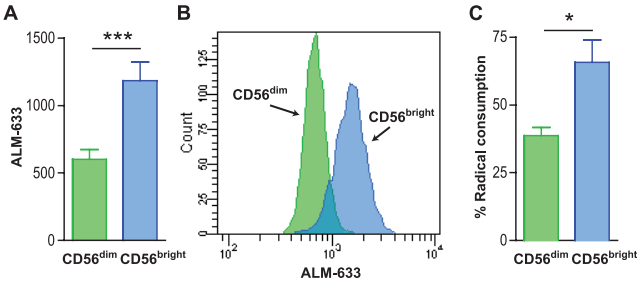

FIGURE 5. CD56^{bright} cells express significantly more surface thiols than CD56^{dim} cells and consume hydrogen peroxide. Data in *A* are the mean ± SEM of MFI values ($p < 0.001$, $n = 4$). *B*, A representative experiment showing thiol-specific binding of Alexa 633 maleimide (ALM-633). *C*, CD56^{bright} cells neutralize exogenous hydrogen peroxide more efficiently than CD56^{dim} cells. Pure populations of the two NK cell subsets were incubated with 30 μM H_2O_2 for 30 min. Remaining hydrogen peroxide was assayed using chemiluminescence. Data are mean ± SEM, $p = 0.03$, $n = 5$. *, $p < 0.05$; ***, $p < 0.001$.

systems are thiols, such as glutathione and thioredoxin (32). Exofacial surface thiols are needed for proper activation of lymphocytes (33, 34), but are also considered to function as a first line of defense against oxidative challenge by neutralizing oxygen radicals. To investigate thiol expression in CD56^{dim} and CD56^{bright} cells, we stained freshly isolated PBMCs for cell-surface thiols using fluorochrome-conjugated maleimide. As shown in Fig. 5, *A* and *B*, the MFI value (which reflects the median number of thiols expressed per cell) for CD56^{bright} cells was approximately twice that of CD56^{dim} cells. Pretreatment with *N*-ethyl maleimide (at 2.5 μM), which blocks cell-surface thiols (29) was found to sensitize CD56^{bright} NK cells to H_2O_2 (data not shown), thus supporting that thiol expression contributed to the resistance of this subset to oxidants.

Finally, we tested whether the higher thiol expression on CD56^{bright} NK cells was accompanied by a higher capacity of neutralizing oxygen radicals. In these experiments, sorted preparations of CD56^{dim} and CD56^{bright} NK cells were exposed to hydrogen peroxide. After incubation for 30 min, the consumption of hydrogen peroxide was determined using isoluminol-ECL. As shown in Fig. 5*C*, CD56^{bright} NK cells consumed hydrogen peroxide significantly more efficiently than CD56^{dim} cells.

Discussion

In recent years, it has become clear that the human NK cell population comprises at least two functionally distinct subsets, the highly cytotoxic CD56^{dim} cells and the less cytotoxic but more efficiently cytokine-producing CD56^{bright} cells (7). A main finding in the present report is that CD56^{bright} NK cells remain viable when exposed to phagocyte-derived or exogenously added hydrogen peroxide, while CD56^{dim} cells rapidly undergo apoptosis. In addition, we show that 1) CD56^{bright} cells express higher levels of cell-surface thiols than CD56^{dim} cells, 2) CD56^{bright} cells are significantly more efficient at neutralizing hydrogen peroxide than CD56^{dim} cells, and 3) CD56^{bright} cells, in contrast to CD56^{dim} cells, retain their capacity to synthesize IFN-γ in conditions of oxidative stress.

Recent findings highlight that oxygen radical-induced inactivation of lymphocytes may constitute a significant mechanism of immunosuppression, with implications for the development of autoimmunity as well as for the function of cytotoxic lymphocytes in malignant diseases. Rodents with mutations in a gene encoding a pivotal component of the phagocyte NADPH oxidase, with an ensuing deficiency of oxygen radical synthesis, display increased susceptibility to arthritis. T cells from these animals show lower threshold for reactivity, suggesting that oxygen radicals participate in controlling autoimmunity and arthritis development (22, 35). Although oxygen radical-induced inhibition of lymphocyte function thus may contribute to protection against autoimmunity, a reverse situation appears to be at hand in malignant diseases. Thus, oxygen radicals, formed by phagocytic cells at the site of malignant expansion, are believed to significantly contribute to the characteristic state of anergy of cytotoxic lymphocytes in, e.g., colorectal cancer, malignant melanoma, renal cell carcinoma, and certain hemopoietic cancers, in addition to chronic viral infections such as HIV and hepatitis C (15–20, 25, 36).

It may be hypothesized that the sensitivity of the highly cytotoxic CD56^{dim} NK cells to oxygen radicals has evolved as an emergency brake for potentially dangerous immune responses, complementing the inhibitory signals transduced by the CD94/NKG2A heterodimer and the killer Ig-like receptor group of receptors on CD56^{dim} cells, and that oxygen radical-mediated inactivation of CD56^{dim} cell may be used by microbes and transformed cells to evade NK cell-mediated cytotoxicity. Regarding CD56^{bright} cells, oxidant-mediated inactivation might not be urgently needed as 1) these cells display low cytotoxicity, and 2) recent data, albeit a subject of discussion, suggest that CD56^{bright} cells are precursors of CD56^{dim} cells (13). The resistance of CD56^{bright} cells to oxygen radical-inflicted toxicity is coherent with earlier reports demonstrating that activating NK cell receptors, such as NKp46 and NKG2D, are retained on the surface of CD56^{bright} cells but down-regulated on CD56^{dim} cells after oxygen radical exposure (37).

A tentative conclusion from the present report is that CD56^{bright} cells, in contrast to CD56^{dim} cells, remain viable and functional also in tissues exposed to oxidative stress, such as acute and chronic bacterial infections, chronic viral infections, chronic inflammation, and in the malignant microenvironment. Although further studies are needed to clarify the functional consequences of the differential regulation of these NK cell subsets, recent reports regarding the distribution of CD56^{bright} and CD56^{dim} cells in such tissues may reflect their differential sensitivity to radical-induced toxicity. Thus, the CD56^{dim}/CD56^{bright} ratio of NK cells is reportedly shifted in favor of the CD56^{bright} subset in pathologies such as head and neck cancer, breast cancer, hepatitis C, tuberculosis, and chronic inflammatory diseases (38–41). It is tempting to speculate that these clinical findings may mirror the scope reported in this study: in the oxidatively challenging environment in these pathologies, the oxygen radical-sensitive CD56^{dim} NK cells succumb, while the resistant CD56^{bright} NK cells survive.

In conclusion, our results demonstrate an unexpected dissimilarity between the two dominant NK cell subsets in that NK cells with CD56^{bright} phenotype are strikingly resistant to oxygen radicals, whereas CD56^{dim} cells are highly prone to undergo apoptosis. The resistance to oxidants of CD56^{bright} cells appears to be explained by a high capacity of neutralizing oxygen radicals, which in turn may be related to a high cell-surface expression of antioxidative thiol groups.

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Disclosures

The authors have no financial conflict of interest.

References

1. Robertson, M. J., and J. Ritz. 1990. Biology and clinical relevance of human natural killer cells. *Blood* 76: 2421–2438.

2. Biron, C. A., K. S. Byron, and J. L. Sullivan. 1989. Severe herpesvirus infections in an adolescent without natural killer cells. *N. Engl. J. Med.* 320: 1731–1735.
3. Cerwenka, A., and L. L. Lanier. 2001. Natural killer cells, viruses and cancer. *Nat. Rev. Immunol.* 1: 41–49.
4. Trinchieri, G., M. Matsumoto-Kobayashi, S. C. Clark, J. Seehra, L. London, and B. Perussia. 1984. Response of resting human peripheral blood natural killer cells to interleukin 2. *J. Exp. Med.* 160: 1147–1169.
5. Martin-Fontecha, A., L. L. Thomsen, S. Brett, C. Gerard, M. Lipp, A. Lanzavecchia, and F. Sallusto. 2004. Induced recruitment of NK cells to lymph nodes provides IFN-γ for Th1 priming. *Nat. Immunol.* 5: 1260–1265.
6. Farag, S. S., and M. A. Caligiuri. 2006. Human natural killer cell development and biology. *Blood Rev.* 20: 123–137.
7. Cooper, M. A., T. A. Fehniger, S. C. Turner, K. S. Chen, B. A. Ghaheri, T. Ghayur, W. E. Carson, and M. A. Caligiuri. 2001. Human natural killer cells: a unique innate immunoregulatory role for the $CD56^{bright}$ subset. *Blood* 97: 3146–3151.
8. Nagler, A., L. L. Lanier, S. Cwirla, and J. H. Phillips. 1989. Comparative studies of human FcRIII-positive and negative natural killer cells. *J. Immunol.* 143: 3183–3191.
9. Frey, M., N. B. Packianathan, T. A. Fehniger, M. E. Ross, W. C. Wang, C. C. Stewart, M. A. Caligiuri, and S. S. Evans. 1998. Differential expression and function of L-selectin on $CD56^{bright}$ and $CD56^{dim}$ natural killer cell subsets. *J. Immunol.* 161: 400–408.
10. Campbell, J. J., S. Qin, D. Unutmaz, D. Soler, K. E. Murphy, M. R. Hodge, L. Wu, and E. C. Butcher. 2001. Unique subpopulations of $CD56^{+}$ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. *J. Immunol.* 166: 6477–6482.
11. Fehniger, T. A., M. A. Cooper, G. J. Nuovo, M. Cella, F. Facchetti, M. Colonna, and M. A. Caligiuri. 2003. $CD56^{bright}$ natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood* 101: 3052–3057.
12. Vitale, M., M. Della Chiesa, S. Carlomagno, C. Romagnani, A. Thiel, L. Moretta, and A. Moretta. 2004. The small subset of $CD56^{bright}CD16^{-}$ natural killer cells is selectively responsible for both cell proliferation and interferon-γ production upon interaction with dendritic cells. *Eur. J. Immunol.* 34: 1715–1722.
13. Ferlazzo, G., D. Thomas, S. L. Lin, K. Goodman, B. Morandi, W. A. Muller, A. Moretta, and C. Munz. 2004. The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. *J. Immunol.* 172: 1455–1462.
14. Morandi, B., G. Bougras, W. A. Muller, G. Ferlazzo, and C. Munz. 2006. NK cells of human secondary lymphoid tissues enhance T cell polarization via IFN-γ secretion. *Eur. J. Immunol.* 36: 2394–2400.
15. Hellstrand, K. 2003. Melanoma immunotherapy: a battle against radicals? *Trends Immunol.* 24: 232–233; author reply 234.
16. Kiessling, R., K. Wasserman, S. Horiguchi, K. Kono, J. Sjoberg, P. Pisa, and M. Petersson. 1999. Tumor-induced immune dysfunction. *Cancer Immunol. Immunother.* 48: 353–362.
17. Kono, K., F. Salazar-Onfray, M. Petersson, J. Hansson, G. Masucci, K. Wasserman, T. Nakazawa, P. Anderson, and R. Kiessling. 1996. Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing ζ molecules and inhibits tumor-specific T cell-and natural killer cell-mediated cytotoxicity. *Eur. J. Immunol.* 26: 1308–1313.
18. Schmielau, J., and O. J. Finn. 2001. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of T-cell function in advanced cancer patients. *Cancer Res.* 61: 4756–4760.
19. Dobmeyer, T. S., S. Findhammer, J. M. Dobmeyer, S. A. Klein, B. Raffel, D. Hoelzer, E. B. Helm, D. Kabelitz, and R. Rossol. 1997. Ex vivo induction of apoptosis in lymphocytes is mediated by oxidative stress: role for lymphocyte loss in HIV infection. *Free Radic. Biol. Med.* 22: 775–785.
20. Farinati, F., R. Cardin, P. Degan, N. De Maria, R. A. Floyd, D. H. Van Thiel, and R. Naccarato. 1999. Oxidative DNA damage in circulating leukocytes occurs as an early event in chronic HCV infection. *Free Radic. Biol. Med.* 27: 1284–1291.
21. Hultqvist, M., P. Olofsson, J. Holmberg, B. T. Backstrom, J. Tordsson, and R. Holmdahl. 2004. Enhanced autoimmunity, arthritis, and encephalomyelitis in mice with a reduced oxidative burst due to a mutation in the Ncf1 gene. *Proc. Natl. Acad. Sci. USA* 101: 12646–12651.
22. Gelderman, K. A., M. Hultqvist, J. Holmberg, P. Olofsson, and R. Holmdahl. 2006. T cell surface redox levels determine T cell reactivity and arthritis susceptibility. *Proc. Natl. Acad. Sci. USA* 103: 12831–12836.
23. Hansson, M., A. Asea, U. Ersson, S. Hermodsson, and K. Hellstrand. 1996. Induction of apoptosis in NK cells by monocyte-derived reactive oxygen metabolites. *J. Immunol.* 156: 42–47.
24. Hansson, M., S. Hermodsson, M. Brune, U. H. Mellqvist, P. Naredi, A. Betten, K. R. Gehlsen, and K. Hellstrand. 1999. Histamine protects T cells and natural killer cells against oxidative stress. *J. Interferon Cytokine Res.* 19: 1135–1144.
25. Mellqvist, U. H., M. Hansson, M. Brune, C. Dahlgren, S. Hermodsson, and K. Hellstrand. 2000. Natural killer cell dysfunction and apoptosis induced by chronic myelogenous leukemia cells: role of reactive oxygen species and regulation by histamine. *Blood* 96: 1961–1968.
26. Thoren, F. B., A. I. Romero, and K. Hellstrand. 2006. Oxygen radicals induce poly(ADP-ribose) polymerase-dependent cell death in cytotoxic lymphocytes. *J. Immunol.* 176: 7301–7307.
27. Hellstrand, K., B. Kjellson, and S. Hermodsson. 1991. Monocyte-induced down-modulation of CD16 and CD56 antigens on human natural killer cells and its regulation by histamine H2-receptors. *Cell. Immunol.* 138: 44–54.
28. Asemissen, A. M., C. Scheibenbogen, A. Letsch, K. Hellstrand, F. Thoren, K. Gehlsen, A. Schmittel, E. Thiel, and U. Keilholz. 2005. Addition of histamine to interleukin 2 treatment augments type 1 T-cell responses in patients with melanoma in vivo: immunologic results from a randomized clinical trial of interleukin 2 with or without histamine (MP 104). *Clin. Cancer Res.* 11: 290–297.
29. Sahaf, B., K. Heydari, L. A. Herzenberg, and L. A. Herzenberg. 2003. Lymphocyte surface thiol levels. *Proc. Natl. Acad. Sci. USA* 100: 4001–4005.
30. Dahlgren, C., and A. Karlsson. 1999. Respiratory burst in human neutrophils. *J. Immunol. Methods* 232: 3–14.
31. Malmberg, K. J., V. Arulampalam, F. Ichihara, M. Petersson, K. Seki, T. Andersson, R. Lenkei, G. Masucci, S. Pettersson, and R. Kiessling. 2001. Inhibition of activated/memory ($CD45RO^{+}$) T cells by oxidative stress associated with block of NF-κB activation. *J. Immunol.* 167: 2595–2601.
32. Winyard, P. G., C. J. Moody, and C. Jacob. 2005. Oxidative activation of antioxidant defence. *Trends Biochem. Sci.* 30: 453–461.
33. Kanner, S. B., T. J. Kavanagh, A. Grossmann, S. L. Hu, J. B. Bolen, P. S. Rabinovitch, and J. A. Ledbetter. 1992. Sulfhydryl oxidation down-regulates T-cell signaling and inhibits tyrosine phosphorylation of phospholipase Cγ1. *Proc. Natl. Acad. Sci. USA* 89: 300–304.
34. Angelini, G., S. Gardella, M. Ardy, M. R. Ciriolo, G. Filomeni, G. Di Trapani, F. Clarke, R. Sitia, and A. Rubartelli. 2002. Antigen-presenting dendritic cells provide the reducing extracellular microenvironment required for T lymphocyte activation. *Proc. Natl. Acad. Sci. USA* 99: 1491–1496.
35. Olofsson, P., J. Holmberg, J. Tordsson, S. Lu, B. Akerstrom, and R. Holmdahl. 2003. Positional identification of Ncf1 as a gene that regulates arthritis severity in rats. *Nat. Genet.* 33: 25–32.
36. Samlowski, W. E., R. Petersen, S. Cuzzocrea, H. Macarthur, D. Burton, J. R. McGregor, and D. Salvemini. 2003. A nonpeptidyl mimic of superoxide dismutase, M40403, inhibits dose-limiting hypotension associated with interleukin-2 and increases its antitumor effects. *Nat. Med.* 9: 750–755.
37. Romero, A. I., F. B. Thoren, M. Brune, and K. Hellstrand. 2006. NKp46 and NKG2D receptor expression in NK cells with $CD56^{dim}$ and $CD56^{bright}$ phenotype: regulation by histamine and reactive oxygen species. *Br. J. Haematol.* 132: 91–98.
38. Bauernhofer, T., I. Kuss, B. Henderson, A. S. Baum, and T. L. Whiteside. 2003. Preferential apoptosis of $CD56^{dim}$ natural killer cell subset in patients with cancer. *Eur. J. Immunol.* 33: 119–124.
39. Dalbeth, N., R. Gundle, R. J. Davies, Y. C. Lee, A. J. McMichael, and M. F. Callan. 2004. $CD56^{bright}$ NK cells are enriched at inflammatory sites and can engage with monocytes in a reciprocal program of activation. *J. Immunol.* 173: 6418–6426.
40. Lin, A. W., S. A. Gonzalez, S. Cunningham-Rundles, G. Dorante, S. Marshall, A. Tignor, C. Ha, I. M. Jacobson, and A. H. Talal. 2004. $CD56^{+dim}$ and $CD56^{+bright}$ cell activation and apoptosis in hepatitis C virus infection. *Clin. Exp. Immunol.* 137: 408–416.
41. Schierloh, P., N. Yokobori, M. Aleman, R. M. Musella, M. Beigier-Bompadre, M. A. Saab, L. Alves, E. Abbate, S. S. de la Barrera, and M. C. Sasiain. 2005. Increased susceptibility to apoptosis of $CD56^{dim}CD16^{+}$ NK cells induces the enrichment of IFN-γ-producing $CD56^{bright}$ cells in tuberculous pleurisy. *J. Immunol.* 175: 6852–6860.