The Journal of Immunology

RESEARCH ARTICLE | OCTOBER 01 2014

Microbe-Specific Unconventional T Cells Induce Human Neutrophil Differentiation into Antigen Cross-Presenting Cells FREE

Martin S. Davey; ... et. al

J Immunol (2014) 193 (7): 3704–3716.
https://doi.org/10.4049/jimmunol.1401018

Microbe-Specific Unconventional T Cells Induce Human Neutrophil Differentiation into Antigen Cross-Presenting Cells

Martin S. Davey,*[,1,2] Matt P. Morgan,*[,†,1] Anna Rita Liuzzi,* Christopher J. Tyler,* Mohd Wajid A. Khan,* Tamas Szakmany,*[,‡] Judith E. Hall,* Bernhard Moser,* and Matthias Eberl*

The early immune response to microbes is dominated by the recruitment of neutrophils whose primary function is to clear invading pathogens. However, there is emerging evidence that neutrophils play additional effector and regulatory roles. The present study demonstrates that human neutrophils assume Ag cross-presenting functions and suggests a plausible scenario for the local generation of APC-like neutrophils through the mobilization of unconventional T cells in response to microbial metabolites. Vγ9/Vδ2 T cells and mucosal-associated invariant T cells are abundant in blood, inflamed tissues, and mucosal barriers. In this study, both human cell types responded rapidly to neutrophils after phagocytosis of Gram-positive and Gram-negative bacteria producing the corresponding ligands, and in turn mediated the differentiation of neutrophils into APCs for both $CD4^+$ and $CD8^+$ T cells through secretion of GM-CSF, IFN-γ, and TNF-α. In patients with acute sepsis, circulating neutrophils displayed a similar APC-like phenotype and readily processed soluble proteins for cross-presentation of antigenic peptides to $CD8^+$ T cells, at a time when peripheral Vγ9/Vδ2 T cells were highly activated. Our findings indicate that unconventional T cells represent key controllers of neutrophil-driven innate and adaptive responses to a broad range of pathogens. ***The Journal of Immunology*****, 2014, 193: 3704–3716.**

Neutrophils are the first cells that are recruited to sites of microbial infection. Although classically viewed as terminally differentiated cells, there is emerging evidence that neutrophils represent key components of the effector and regulatory arms of the innate and adaptive immune system (1–3). As such, neutrophils regulate the recruitment and function of various cell types and interact with immune and nonimmune cells. Intriguingly, neutrophils directly affect Ag-specific responses by facilitating monocyte differentiation and dendritic cell maturation, and by interacting with T cells and B cells (4–10). Murine neutrophils have been shown to present Ags to both $CD4^+$ and $CD8^+$ T cells (11–13), and to differentiate into neutrophil–dendritic cell hybrids in vitro and in vivo (14, 15). In humans, neutrophils with a phenotype consistent with a possible APC function, including expression of MHC class II, have been found in diverse inflammatory and infectious conditions (16–22). This notwithstanding, direct Ag presentation by neutrophils has to date not been demonstrated in patients, especially with respect to an induction of Ag-specific $CD8^+$ T cell responses upon cross-presentation of exogenous proteins.

The physiological context underlying the differentiation of neutrophils into APCs and the implications for Ag-specific immune responses remain unclear. Unconventional T cells such as human γδ T cells, NKT cells, and mucosal-associated invariant T (MAIT) cells represent unique sentinel cells with a distinctive responsiveness to low m.w. compounds akin to pathogen and danger-associated molecular patterns (23–25). Such unconventional T cells represent a substantial proportion of all T cells in blood and mucosal epithelia, accumulate in inflamed tissues, and constitute an efficient immune surveillance network in inflammatory and infectious diseases as well as in tumorigenesis. Besides orchestrating local responses by engaging with other components of the inflammatory infiltrate (26–29), unconventional T cells are also ideally positioned in lymphoid tissues to interact with freshly recruited monocytes and neutrophils (30–32). We previously showed that human γδ T cells enhance the short-term survival of neutrophils but did not characterize these surviving neutrophils on a phenotypical and functional level (28). In this work, we studied the outcome of such a crosstalk of human neutrophils with both γδ T cells and MAIT cells in vitro and translated our findings to patients with severe sepsis. We demonstrate that neutrophils with APC-like features can be found in blood during acute infection,

*Cardiff Institute of Infection and Immunity, School of Medicine, Cardiff University, Cardiff CF14 4XN, United Kingdom; †Cardiff and Vale University Health Board, Cardiff CF14 4XW, United Kingdom; and ‡Cwm Taf University Health Board, Llantrisant CF72 8XR, United Kingdom

[1]M.S.D. and M.P.M. contributed equally to this study.

[2]Current address: Birmingham Cancer Research UK Centre, School of Cancer Sciences, University of Birmingham, Birmingham, U.K.

Received for publication April 21, 2014. Accepted for publication July 28, 2014.

This work was supported by the United Kingdom Clinical Research Network Study Portfolio, the National Institute for Social Care and Health Research (NISCHR), the NISCHR/Wellcome Trust Institutional Strategic Support Fund, the Severnside Alliance for Translational Research/South East Wales Academic Health Science Partnership Health Technology Challenge Scheme, the European Union-Framework Programme 7 Marie Curie Initial Training Network "European Training and Research in Peritoneal Dialysis," a Medical Research Council Ph.D. studentship (to C.J.T.), and Cancer Research UK.

Address correspondence and reprint requests to Dr. Matthias Eberl, Cardiff Institute of Infection and Immunity, Henry Wellcome Building, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, U.K. E-mail address: eberlm@cf.ac.uk

The online version of this article contains supplemental material.

Abbreviations used in this article: DMRL, 6,7-dimethyl-8-D-ribityllumazine; HMB-PP, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; MAIT, mucosal-associated invariant T; MR1, MHC-related protein 1; PPD, *Mycobacterium tuberculosis* purified protein derivate; SIRS, systemic inflammatory response syndrome; sTNFR, soluble TNFR; TSST-1, *Staphylococcus aureus* toxic shock syndrome toxin-1.

and that the phenotype and ex vivo function of circulating sepsis neutrophils was replicated in vitro upon priming of neutrophils by human γδ T cells and MAIT cells. Our findings thus provide a possible physiological context and propose a cellular mechanism for the local generation of neutrophils with APC functions, including their potential to cross-present soluble Ags to $CD8^+$ T cells, in response to a broad range of microbial pathogens.

Materials and Methods

Subjects

This study was approved by the South East Wales Local Ethics Committee under reference numbers 08/WSE04/17 and 10/WSE04/21 and conducted according to the principles expressed in the Declaration of Helsinki and under local ethical guidelines. Sampling of adult patients with sterile systemic inflammatory response syndrome (SIRS) or with acute sepsis (defined as patients with SIRS in conjunction with a proven or suspected infection) was carried out within the United Kingdom Clinical Research Network under study portfolio UKCRN ID 11231, "Cellular and Biochemical Investigations in Sepsis." All study participants provided written informed consent for the collection of samples and their subsequent analysis. A waiver of consent system was used when patients were unable to provide prospective informed consent due to the nature of their critical illness or therapeutic sedation at the time of recruitment. In all cases, retrospective informed consent was sought as soon as the patient recovered and regained capacity. In cases in which a patient died before regaining capacity, the initial consultee's approval would stand.

Sepsis patients had a proven infection as confirmed by positive culture of at least one relevant sample according to the local microbiology laboratory overseen by Public Health Wales, and developed at least three of the four following SIRS criteria over the previous 36 h: 1) temperature from any site >38°C or core <36°C; 2) heart rate of >90 beats/min (unless individual had a medical condition or was receiving treatment preventing tachycardia); 3) respiratory rate of >20 breaths/min, arterial $PaCO_2$ <32 mmHg, or mechanical ventilation for an acute process; and 4) total WBC >12,000 cells/mm^3 or <4,000 cells/mm^3 or differential WBC count showing >10% immature (band) forms (n = 37; age range 35–82 y, median 63 y; 51% female). Patients with sterile SIRS developed at least three of the four SIRS criteria but had no suspected or proven microbial infection (n = 14; age range 26–70 y, median 48 y; 21% female). All patients with sepsis or SIRS had at least one documented organ failure on recruitment to the study and were either mechanically ventilated, on inotropic support, or received acute renal replacement therapy. Healthy donors served as controls for the patient cohorts (n = 10; age range 31–68 y, median 59 y; 20% female). Individuals were excluded from the study if pregnant or breastfeeding; suffering from documented severe immune deficiency or severe liver failure; admitted postcardiac arrest; treated with high-dose steroids or immunosuppressant drugs for the last 6 mo; or unlikely to survive for the duration of the study period regardless of treatment.

Media, reagent, and Abs

Culture medium was RPMI 1640 medium supplemented with 2 mM L-glutamine, 1% sodium pyruvate, 50 μg/ml penicillin/streptomycin, and 10% FCS (Invitrogen). Synthetic (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) was provided by H. Jomaa (Justus-Liebig University Giessen); synthetic 6,7-dimethyl-8-D-ribityllumazine (DMRL) was provided by B. Illarionov (Hamburg School of Food Science). *Staphylococcus aureus* toxic shock syndrome toxin-1 (TSST-1) was purchased from Toxin Technology; *Mycobacterium tuberculosis* purified protein derivate (PPD) was purchased from Statens Serum Institut (Copenhagen, Denmark). *Salmonella abortus equi* LPS, brefeldin A, and BSA-FITC were purchased from Sigma-Aldrich. Recombinant IFN-γ, TNF-α, and GM-CSF was purchased from Miltenyi Biotec. Human T-activator CD3/CD28 Dynabeads, CFSE, and 10-kDa dextran-FITC were purchased from Life Technologies.

The following mAbs were used for surface labeling: anti-CD3 (UCHT1, SK7, HIT3a), anti-CD4 (SK3, RPA-T4), anti-CD8 (SK1, HIT8a, RPA-T8), anti-CD11c (S-HCL-3), anti-CD14 (M5E2, MOP9), anti-CD15 (HI98), anti-CD16 (3G8), anti-CD25 (M-A251), anti-CD27 (M-T271), anti-CD31 (WM-59), anti-CD32 (FLI8.26), anti-CD45RO (UCHL1), anti-CD49d (9F10), anti-CD50 (TU41), anti-CD54 (HA58), anti-CD56 (B159), anti-CD62L (DREG-56), anti-CD64 (10.1), anti-CD69 (FN50), anti-CD70 (Ki24), anti-CD71 (M-A712), anti-CD72 (J4-112), anti-CD83 (HB15e), anti-CD86 (2331), anti-CD209 (DCN46), anti–HLA-DR (L243), anti–TCR-Vδ2 (B6.1), anti-CCR4 (1G1), anti-CCR5 (2D7), anti-CCR7 (3D12), and anti-CXCR3 (1C6) from BD Biosciences; anti–TCR-Vβ2 (MPB2D5), anti–TCR-Vγ9 (Immu360), and anti-CD40 (mAB89) from Beckman Coulter; anti-CD11a (HI111), anti-CD66b (G10F5), anti-CD154 (24-31), anti-CD161 (HP-3G10), anti–HLA-ABC (w6/32), and anti–TCR-Vα7.2 (3C10) from BioLegend; anti-CD11b (ICRF44), anti-CD14 (61D3), anti-CD19 (SJ25C1), anti-CD25 (BC96), anti-CD45RA (HI100), and anti-CD80 (2D10.4) from eBioscience; anti–HLA-A2 (BB7.2) from Serotec; and anti-CCR9 (248601) and anti-CCR10 (314305) from R&D Systems; together with appropriate isotype controls. Intracellular cytokines were detected using anti–IFN-γ (B27, BD Biosciences; 4S.B3, eBioscience) and anti–TNF-α (6401.1111, BD Biosciences; 188, Beckman Coulter). Blocking reagents used included anti-Vα7.2 (3C10; BioLegend); anti–TCR-Vγ9 (Immu360; Beckman Coulter); anti-TLR4 (HTA125; eBioscience); anti-CD277 (103.2; D. Olive, Université de la Méditerranée, Marseille, France); anti–MHC-related protein 1 (MR1) (26.5; T. Hansen, Washington University School of Medicine, St. Louis, MO); anti–IFN-γ (25718) and anti–GM-CSF (3209) (BioLegend); and soluble TNFR (sTNFR) p75-IgG1 fusion protein (etanercept/Enbrel; Amgen).

Cells

Total leukocytes from healthy donors and patients were isolated from heparinized blood by mixing with HetaSep (StemCell Technologies), followed by sedimentation of RBCs (Supplemental Fig. 1A). Neutrophils were purified from whole blood or Lymphoprep (Axis-Shield) separated granulocytes by HetaSep sedimentation, followed by negative selection using the EasySep neutrophil enrichment kit (StemCell Technologies) (33), resulting in purities of >99.2% $CD14^-CD66b^+CD15^+$ and <0.1% contaminating monocytes (Supplemental Fig. 1B). Total $CD3^+$ T cells (>98%) were isolated from PBMC using the pan T cell isolation kit II (Miltenyi Biotec); $CD4^+$ and $CD8^+$ T cells (>98%) were obtained using the corresponding EasySep kits (StemCell Technologies). $Vγ9^+$ T cells (>98%) were purified using anti–Vγ9-PE-Cy5 (Immu360; Beckman Coulter) and anti-PE microbeads (Miltenyi Biotec); $Vα7.2^+$ T cells (>98%) were purified using anti–Vα7.2-PE (3C10; BioLegend) and anti-PE microbeads. Alternatively, $Vγ9^+$ $CD3^+$ γδ T cells or $Vα7.2^+$ $CD161^+$ $CD3^+$ MAIT cells were sorted to purities >99% using a FACSAria II (BD Biosciences).

Bacteria

Clinical isolates of *Enterobacter cloacae*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *S. aureus* (28) were grown in liquid Luria-Bertani broth and on solid Columbia blood agar (Oxoid). The distribution of the nonmevalonate and riboflavin pathways across microbial species was determined based on the absence or presence of the enzymes HMB-PP synthase (EC 1.17.7.1) and DMRL synthase (EC 2.5.1.78) in the corresponding genomes, according to the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg).

T cell culture

PBMC were stimulated with 0.1–100 nM HMB-PP or 0.1–100 μM DMRL. $Vγ9^+$ T cells or $Vα7.2^+$ T cells were cocultured with autologous monocytes at a ratio of 1:1, in the presence of 25% (v/v) cell-free supernatants from neutrophils that had phagocytosed live bacteria, as described previously (28). For blocking experiments, anti–TCR-Vα7.2, anti–TCR-Vγ9, anti-CD277, and anti-MR1 were used at 1–20 μg/ml.

Neutrophil culture

Freshly isolated neutrophils were cultured for up to 48 h in the absence or presence of autologous Vγ9/Vδ2 T cells or MAIT cells at a ratio of 10:1, and 10 nM HMB-PP or anti-CD3/CD28 dynabeads (1 bead per T cell). Alternatively, neutrophils were cultured with 25–50% (v/v) conditioned medium obtained from purified Vγ9/Vδ2 T cells or MAIT cells stimulated for 24 h with anti-CD3/CD28 dynabeads (1 bead per cell) or 100 nM HMB-PP. Other stimuli included 100 ng/ml LPS and 100 U/ml recombinant IFN-γ, TNF-α, and/or GM-CSF. sTNFR p75-IgG1 fusion protein, anti–IFN-γ, and anti–GM-CSF were used at 10 μg/ml. Neutrophil survival and activation were assessed by flow cytometry, after gating on $CD15^+$ cells and exclusion of $Vγ9^+$ or $Vα7.2^+$ cells where appropriate. For morphological analyses, neutrophils were centrifuged onto cytospin slides, stained with May-Grünwald-Giemsa solution, and analyzed by light microscopy.

Functional assays

Endocytosis and APC functions were assessed as before (34–38). Freshly purified neutrophils and neutrophils cultured for 24 h in the presence or absence of unconventional T cell–conditioned medium were incubated with 500 μg/ml 10-kDa dextran-FITC or BSA-FITC for up to 60 min at 4°C or 37°C. Endocytic uptake was measured immediately by flow cytom-

etry; the specific uptake of each reagent was calculated by subtracting the background MFI at 4°C from the MFI obtained at 37°C.

For MHC class II–restricted presentation of Ags, activated neutrophils were generated by 48-h culture with a combination of IFN-γ, GM-CSF, and/or TNF-α, or with unconventional T cell–conditioned medium. Neutrophils were pulsed with 10 ng/ml TSST-1 for 1 h. After extensive washing, neutrophils were mixed with autologous $CD4^+$ T cells at a ratio of 1:1; 1 h later 10 μg/ml brefeldin A was added and cultures were incubated for an additional 4 h. Activation of TSST-1–responsive $V\beta2^+$ $CD4^+$ T cells was assessed by intracellular cytokine staining and analysis by flow cytometry (35). To assess $CD4^+$ and $CD8^+$ T cell responses to complex Ag preparations, neutrophils were pulsed with 1–10 μg/ml PPD for the last 18 h of the 48-h culture phase. After extensive washing, neutrophils were mixed with CFSE-labeled autologous $CD3^+$ T cells at a ratio of 1:1 and incubated for 7 d. CFSE dilution in the $CD4^+$ and $CD8^+$ T cell populations was assessed by flow cytometry, after exclusion of $CD66b^+$ cells.

For MHC class I–restricted Ag presentation, Ag-specific HLA-A2–restricted $CD8^+$ T cell lines were generated using the immunodominant peptide of influenza matrix protein, M1(p58–66) (GILGFVFTL), as described before (37, 38). M1(p58–66)-specific responder $CD8^+$ T cells used in APC assays were >95% pure, as confirmed by tetramer staining (data not shown). Activated neutrophils from $HLA\text{-}A2^+$ donors were generated as above, using unconventional T cell–conditioned medium or recombinant cytokines, and pulsed for 1 h with 0.1 μM peptide. For cross-presentation assays, 0.01–1 μM recombinant influenza M1 protein was added during the last 18 h of the 48-h neutrophil culture period. Fresh neutrophils from $HLA\text{-}A2^+$ sepsis patients were incubated with 0.01–1 μM recombinant influenza M1 protein for 18 h or cultured in medium for 17 h prior to addition of 0.1 μM M1(p58–66) peptide for an additional 1 h. In each case, following extensive washing, neutrophils were incubated with $HLA\text{-}A2^+$ peptide-specific $CD8^+$ T cells at a ratio of 1:1; after 1 h, 10 μg/ml brefeldin was added and cultures were incubated for an additional 4 h. Activation of $CD8^+$ T cells was assessed by intracellular cytokine staining and analyzed by flow cytometry, after exclusion of $CD66b^+$ cells.

Flow cytometry

Cells were acquired on an eight-color FACSCanto II (BD Biosciences) and analyzed with FlowJo (Tree Star). Single cells of interest were gated based on their appearance in side and forward scatter area/height, exclusion of live/dead staining (fixable Aqua; Invitrogen), and surface staining. Apoptotic cells were identified using annexin-V (BD Biosciences).

ELISA

Cell culture supernatants were analyzed on a Dynex MRX II reader, using ELISA kits for IL-17A (R&D Systems) as well as IFN-γ and TNF-α (eBioscience). Cell-free plasma samples and unconventional T cell–conditioned media were analyzed on a SECTOR Imager 6000 using the ultrasensitive human proinflammatory 9-plex kit (Meso Scale Discovery).

Statistics

Data were analyzed using two-tailed Student *t* tests for normally distributed data and Mann–Whitney tests for nonparametric data (GraphPad Prism). Differences between groups were analyzed using one-way ANOVA with Bonferroni's posttests or with Kruskal–Wallis and Dunn's posttests; two-way ANOVA was used when comparing groups with independent variables.

Results

Unconventional human T cells respond to neutrophil-released microbial metabolites

$V\gamma9/V\delta2^+$ γδ T cells recognize the isoprenoid precursor HMB-PP, which is produced via the nonmevalonate pathway by a broad range of Gram-negative and Gram-positive bacteria (27, 39). $V\alpha7.2^+$ $CD161^+$ MAIT cells show a very similar responsiveness to an overlapping, but distinct spectrum of microorganisms by sensing intermediates of the microbial vitamin B2 biosynthesis (Table I) (40–43). We therefore sought to investigate the antimicrobial responses of these two types of unconventional T cells side by side. In this study, Vγ9/Vδ2 T cells, but not MAIT cells, responded to HMB-PP, as judged by induction of CD69 expression (Fig. 1A). In contrast, the riboflavin precursor DMRL induced a dose-dependent activation of MAIT cells, but not Vγ9/Vδ2 T cells. Blocking experiments confirmed a requirement for butyrophilin 3A/CD277 for Vγ9/Vδ2 T cells and the MHC-related protein MR1 for MAIT cells (Fig. 1B), in support of current models of Ag recognition (41–45).

We previously identified a crucial role for neutrophils in facilitating access to HMB-PP by Vγ9/Vδ2 T cells (28). As control, purified $V\gamma9^+$ T cells readily responded to neutrophils after phagocytosis of clinically relevant bacteria, in accordance with the distribution of the nonmevalonate pathways across the different pathogens (Table I). Strikingly, purified $V\alpha7.2^+$ T cells showed very similar responses depending on the utilization of the riboflavin biosynthesis pathway by the phagocytosed species. Activated $V\gamma9^+$ T cells and $V\alpha7.2^+$ T cells upregulated CD69 (Fig. 1C) and secreted IFN-γ (Fig. 1D), but not IL-17A (data not shown). The response of $V\alpha7.2^+$ T cells to microbial compounds was confined to the $CD161^+$ bona fide MAIT cell population (Fig. 1C, 1D). Both Vγ9/Vδ2 T cells and MAIT cells failed to respond to neutrophil-released microbial compounds in the presence of anti-CD277 and anti-MR1, respectively (Fig. 1D), and in the absence of autologous monocytes (Fig. 1E), highlighting a requirement for presentation by accessory cells. These findings reveal a remarkable similarity in the responsiveness of Vγ9/Vδ2 T cells and MAIT cells to microbial metabolites.

Patients with acute sepsis caused by HMB-PP–producing pathogens display elevated levels of activated γδ T cells

To resolve the existence of APC-like neutrophils in human infectious disease and determine a possible link with antimicrobial unconventional T cell responses, we recruited adult patients with newly diagnosed severe sepsis and characterized their circulating leukocytes phenotypically and functionally. As proof of principle for the involvement of unconventional T cells in early inflammatory responses, patients with acute sepsis revealed a substantial activation of Vγ9/Vδ2 T cells, as judged by CD69 expression, but not SIRS patients who served as noninfected controls (Fig. 1F, Supplemental Fig. 1A). Of note, we found a significant increase in the absolute counts and the proportion of Vγ9/Vδ2 T cells among all circulating T cells between patients with microbiologically confirmed infections caused by HMB-PP–producing as opposed to HMB-PP–deficient species (Fig. 1F). These clinical findings evoke earlier studies in patients with acute peritonitis (28) and further support the notion of a differential responsiveness of unconventional T cells to defined pathogen groups that can be detected both locally at the site of infection (46) and systemically in blood (Fig. 1F).

Unconventional human T cells induce prolonged neutrophil survival and activation

We recently showed that Vγ9/Vδ2 T cells trigger short-term (<20 h) survival of autologous neutrophils (28). In this study, highly purified neutrophils cocultured for extended periods with activated Vγ9/Vδ2 T cells or MAIT cells displayed a prolonged survival, as judged by exclusion of amine reactive dyes and retention of surface CD16 (FcγRIII) for at least 48 h (Fig. 2A). A similar effect was observed when incubating purified neutrophils with Vγ9/Vδ2 T cell or MAIT cell–conditioned culture supernatants, indicating a significant contribution of soluble factors in mediating the observed effects (Fig. 2B). In contrast to the highly active metabolite HMB-PP as specific activator of Vγ9/Vδ2 T cells, the MAIT cell activator used in the current study, DMRL, only possesses a relatively modest bioactivity. The true MAIT cell activator is far more potent than DMRL and active at subnanomolar concentrations, but not commercially available and difficult to synthesize chemically (41, 43). Most stimulation experiments with purified MAIT cells were therefore conducted with anti-CD3/CD28–coated beads. Importantly, use of either anti-CD3/CD28 beads or HMB-PP to

Table I. Distribution across clinically relevant microbial pathogens of key biosynthetic pathways that produce metabolites targeted by human unconventional T cells

	Nonmevalonate Pathway (Vγ9/Vδ2 T Cell Activation)	Vitamin B2 Synthesis (MAIT Cell Activation)
Gram-negative bacteria		
Acinetobacter baumannii	+	+
Chryseobacterium gleum	−	+
Enterobacter cloacae	+	+
Escherichia coli	+	+
Haemophilus influenzae	+	+
Helicobacter pylori	+	+
Klebsiella pneumoniae	+	+
Legionella pneumophila	−	+
Neisseria meningitidis	+	+
Pseudomonas aeruginosa	+	+
Shigella dysenteriae	+	+
Gram-positive bacteria		
Bacillus anthracis	+	+
Clostridium difficile	+	+
Corynebacterium diphtheriae	+	+
Enterococcus faecalis	−	−
Listeria monocytogenes	+	−
Mycobacterium tuberculosis	+	+
Propionibacterium acnes	+	+
Staphylococcus aureus	−	+
Streptococcus pyogenes	−	−
Other bacteria		
Borrelia burgdorferi	−	−
Leptospira interrogans	+	+
Mycoplasma genitalium	−	−
Mycoplasma penetrans	+	−
Treponema pallidum	−	−
Yeasts, fungi		
Aspergillus fumigatus	−	+
Candida albicans	−	+
Crytococcus neoformans	−	+
Saccharomyces cerevisiae	−	+

activate Vγ9/Vδ2 T cells elicited identical neutrophil responses (Fig. 2A–C and data not shown). Surviving neutrophils possessed a highly activated morphology, as judged by the presence of hypersegmented nuclei (Fig. 2C). The antiapoptotic effect of unconventional T cells was confirmed by the preservation of the total number of neutrophils present after 48 h of culture and the lack of annexin-V binding (Fig. 2D). As confirmation of their activated status, surviving neutrophils showed pronounced upregulation of CD11b and CD66b expression and complete loss of CD62L (Fig. 2E).

Unconventional T cell–primed neutrophils have a unique APC-like phenotype

Circulating neutrophils in healthy people do not express CD40, CD64 (FcγRI), CD83, or HLA-DR, yet all these surface markers were found on unconventional T cell–primed neutrophils (Fig. 2F). Moreover, these neutrophils also showed a marked upregulation of CD54 (ICAM-1) and HLA-ABC (Fig. 2F), suggestive of a possible function of unconventional T cell–primed neutrophils as APCs for both $CD4^+$ and $CD8^+$ T cells.

The chemokine receptors CCR7, CCR9, and CCR10 remained undetectable under those culture conditions (data not shown), arguing against trafficking of APC-like neutrophils to noninflamed lymph nodes, the intestine, or the skin. In contrast, APC-like neutrophils displayed enhanced expression levels of CXCR3 and CCR4 (data not shown), indicative of an increased responsiveness to inflammatory chemokines and supporting a local role during acute inflammation.

Neutrophils stimulated with defined microbial compounds on their own, in the absence of Vγ9/Vδ2 T cells or MAIT cells, failed to acquire a similar phenotype. Most notably, neutrophils cultured for 48 h in the presence of LPS did not show increased levels of HLA-ABC, HLA-DR, CD40, CD64, or CD83 compared with neutrophils cultured in medium alone (data not shown), emphasizing the crucial and nonredundant contribution of unconventional T cells and their specific ligands to the acquisition of APC characteristics by neutrophils.

Circulating neutrophils in sepsis patients display an APC-like phenotype

To resolve the existence of APC-like neutrophils in human infectious disease, we characterized circulating leukocytes in sepsis patients as a means to access neutrophils that had recently been activated in different infected tissues. Sepsis neutrophils displayed a strikingly altered phenotype compared with neutrophils from healthy individuals and SIRS patients and were characterized by markedly higher expression of CD40, CD64, and CD86 (Fig. 3A). We also found increased surface levels of CD83 and HLA-DR on circulating neutrophils in some patients with sepsis, although this was not significant across the cohort as a whole. Of note, there was a correlation between the expression of CD64 and HLA-DR on sepsis neutrophils, supporting a link between neutrophil activation and APC phenotype (Fig. 3B). These findings indicate the presence of APC-like neutrophils in sepsis patients, despite the generally presumed immune suppression in those individuals, as judged by reduced HLA-DR expression levels on monocytes (data not shown) (47).

Neutrophil survival and APC marker expression are mediated via unconventional T cell–secreted cytokines

To identify the unconventional T cell–derived factor(s) exerting the observed effects on neutrophils, we quantified proinflammatory

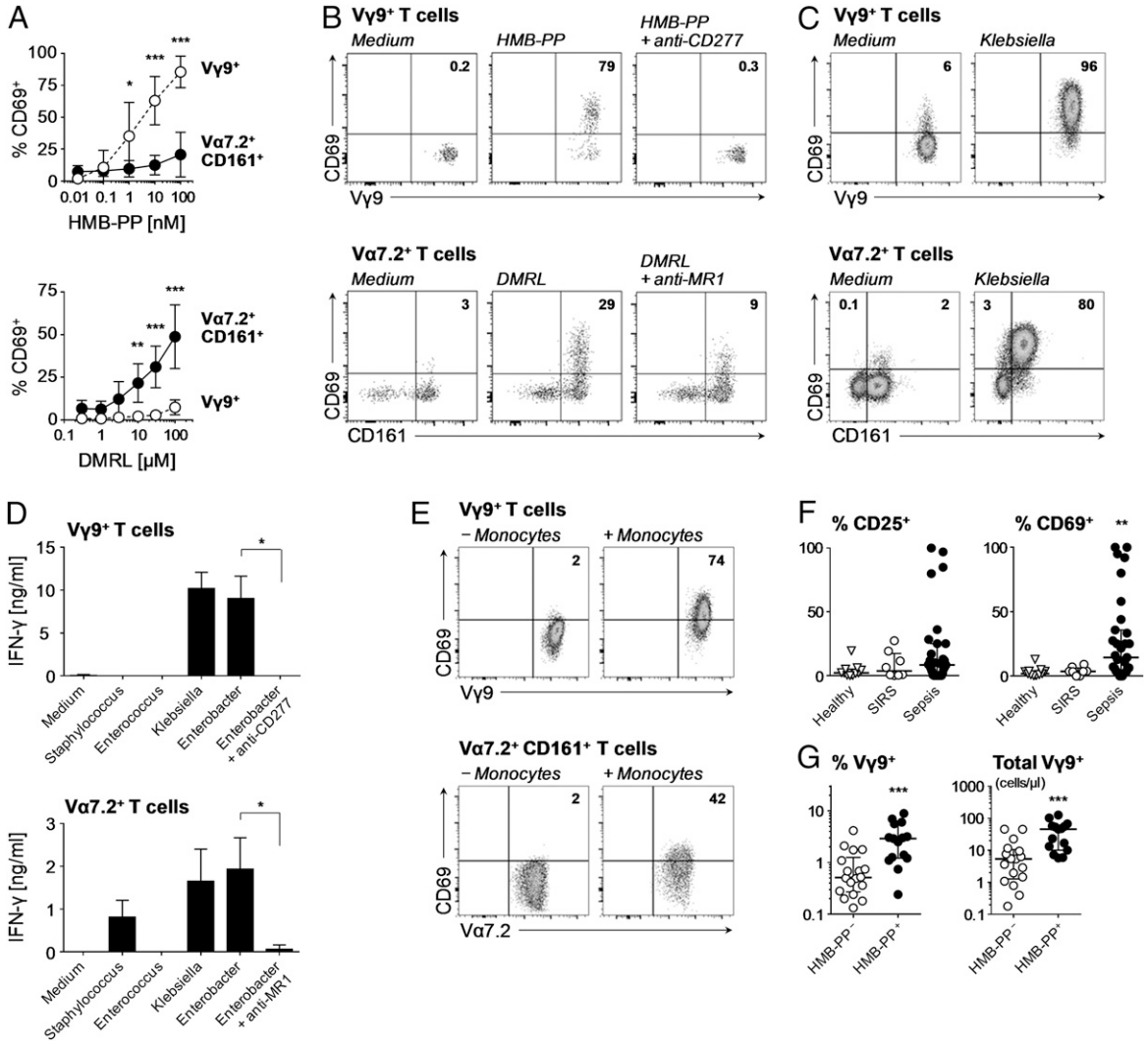

FIGURE 1. Unconventional human T cell responses to microbial metabolites in vitro and in vivo. (**A**) CD69 surface expression by Vγ9$^+$ T cells and Vα7.2$^+$ CD161$^+$ T cells in PBMC stimulated overnight with HMB-PP or DMRL (means ± SD, n = 5). Data were analyzed by two-way ANOVA with Bonferroni's post hoc tests. (**B**) Representative FACS plots of two donors showing CD69 expression by Vγ9$^+$ T cells and Vα7.2$^+$ T cells in PBMC stimulated overnight with 100 nM HMB-PP or 100 μM DMRL, in the absence or presence of anti-CD277 or anti-MR1 mAb. (**C**) CD69 expression by MACS-purified Vγ9$^+$ T cells or Vα7.2$^+$ T cells cocultured overnight with autologous monocytes in the presence of supernatants from neutrophils after phagocytosis of *Klebsiella pneumoniae* (representative of three donors). (**D**) IFN-γ secretion by MACS-purified Vγ9$^+$ T cells or Vα7.2$^+$ T cells cocultured overnight with autologous monocytes in the presence of supernatants from neutrophils after phagocytosis of different bacteria: HMB-PP$^-$ DMRL$^+$, *Staphylococcus aureus*; HMB-PP$^-$ DMRL$^-$, *Enterococcus faecalis*; and HMB-PP$^+$ DMRL$^+$, *Enterobacter cloacae* and *K. pneumoniae* (means ± SD, n = 3–4 donors). Differences between mAb-treated and untreated cultures were analyzed using Mann–Whitney tests. (**E**) CD69 expression by FACS-sorted Vγ9$^+$ T cells or Vα7.2$^+$ CD161$^+$ T cells cocultured overnight with or without autologous monocytes in the presence of supernatants from neutrophils after phagocytosis of *E. cloacae* (representative of two donors). (**F**) Surface expression by CD25 and CD69 on circulating Vγ9$^+$ T cells in healthy controls and in patients with SIRS or sepsis. Each data point represents an individual; lines and error bars depict medians and interquartile ranges. Data were analyzed using Kruskal–Wallis tests and Dunn's multiple comparison tests; comparisons were made with sepsis patients. (**G**) Proportion of Vγ9$^+$ T cells among all circulating T cells and absolute counts of circulating Vγ9$^+$ T cells (in cells/μl blood) in sepsis patients with microbiologically confirmed infections caused by HMB-PP–producing (*E. coli*, *Enterobacter aerogenes*, *Haemophilus influenzae*, *K. pneumonia*e, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, anaerobic Gram-negative bacilli, diphtheroid bacteria) or HMB-PP–deficient organisms (*Aspergillus fumigatus*, *Candida spp.*, *Staphylococcus spp.*, *Streptococcus pneumoniae*). Data were analyzed using Mann–Whitney tests. Differences were considered significant as indicated: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

mediators in the culture supernatants. These experiments revealed a dominant production (>1000 pg/ml on average) of GM-CSF, IFN-γ, and TNF-α by activated Vγ9/Vδ2 T cells and MAIT cells, but only very low levels (<25 pg/ml) of IL-1β, IL-6, and CXCL8, indicating that both unconventional T cell populations share a similar cytokine profile (Fig. 4A). Experiments using blocking reagents identified an involvement of GM-CSF, IFN-γ, and TNF-α in promoting neutrophil survival by both Vγ9/Vδ2 T cells and MAIT cells (Fig. 4B). Whereas neutralization of each individual cytokine on its own had a partial effect, combined blocking of GM-CSF and IFN-γ was most effective in inhibiting neutrophil survival, with blocking of TNF-α having little additive effect. In contrast, CD66b upregulation was mainly triggered by TNF-α (Fig. 4B). Of note, the effect of unconventional T cells on neutrophils could be mimicked in part by using recombinant GM-CSF, IFN-γ, and TNF-α. In this respect, only neutrophils cultured with a combination of all three cytokines exhibited a morphology characterized by hypersegmented nuclei (Fig. 4C). TNF-α was particularly important for the induction of CD40, CD54, CD66b, and MHC class I expression (Fig. 4D). Taken together,

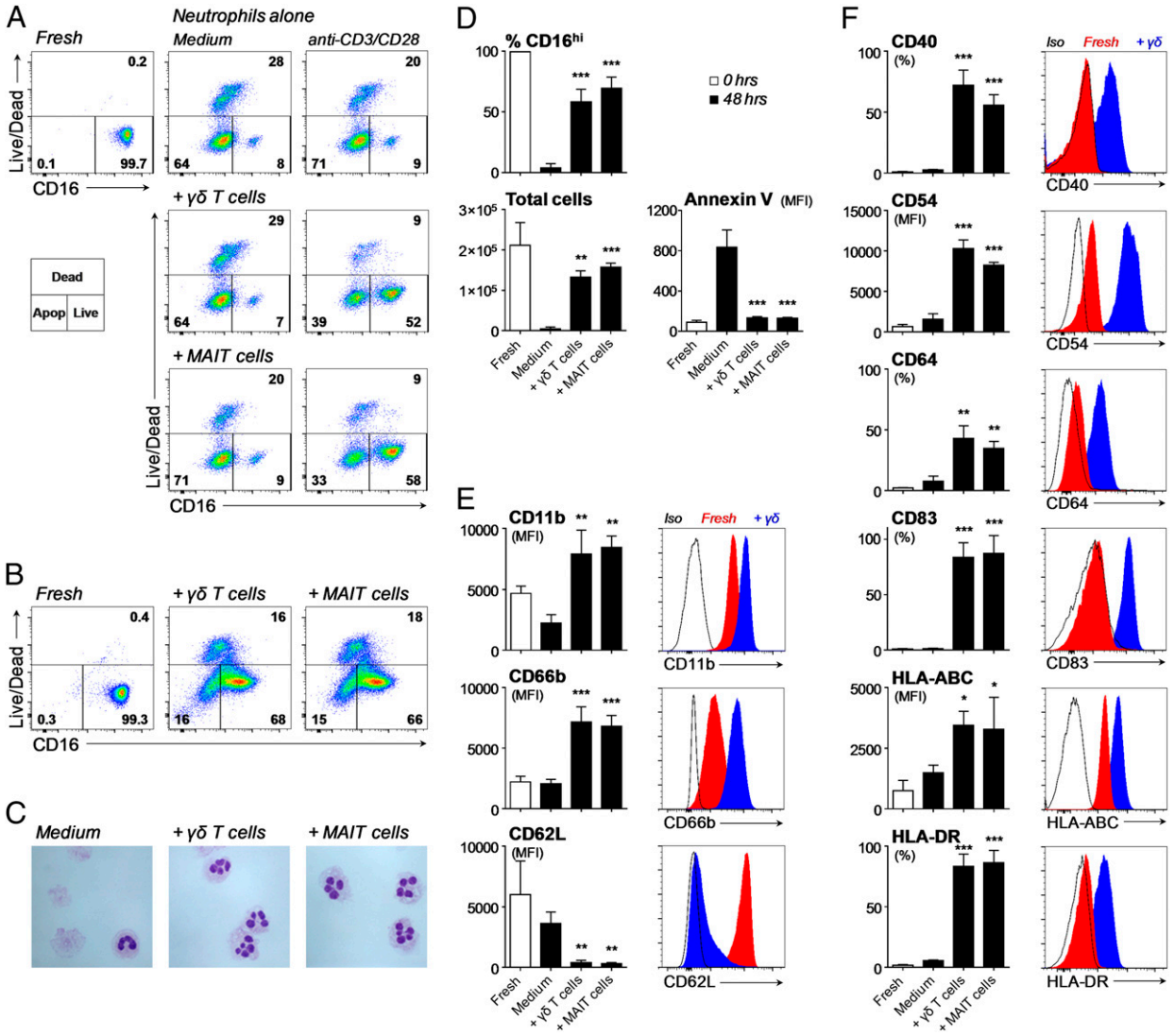

FIGURE 2. Survival, activation, and expression of APC markers by unconventional T cell–primed neutrophils. (**A**) Neutrophil survival judged by retention of CD16 expression and exclusion of live/dead staining after 48-h coculture with FACS-sorted Vγ9/Vδ2 T cells or MAIT cells, in the absence or presence of anti-CD3/CD28 beads. FACS plots are representative of three donors and depict total neutrophils after gating on $CD15^+$ $Vγ9^-$ or $CD15^+$ $Vα7.2^-$ cells. (**B**) Neutrophil survival after 48-h culture in the presence of HMB-PP–activated Vγ9/Vδ2 T cell or anti-CD3/CD28–activated MAIT cell–conditioned medium (representative of three donors). (**C**) Morphological analysis of surviving neutrophils after 48-h culture in the absence or presence of HMB-PP–activated Vγ9/Vδ2 T cell or anti-CD3/CD28–activated MAIT cell–conditioned medium (representative of two donors). Original magnification ×400. (**D**) Neutrophil survival after 48-h culture in the absence or presence of HMB-PP–activated Vγ9/Vδ2 T cell or anti-CD3/CD28–activated MAIT cell–conditioned medium. Shown are means ± SD for the proportion of $CD16^{high}$ cells (n = 9–10), the total number of neutrophils (n = 3), and annexin V staining on $CD16^{high}$ neutrophils (n = 3). Expression of (**E**) activation markers and (**F**) APC markers on freshly isolated neutrophils and $CD16^{high}$ neutrophils after 48-h culture in the absence or presence of HMB-PP–activated Vγ9/Vδ2 T cell– or anti-CD3/CD28–activated MAIT cell–conditioned medium. Data shown are means ± SD and representative histograms from three individual donors. Data were analyzed by one-way ANOVA with Bonferroni's post hoc tests; comparisons were made with medium controls. Differences were considered significant as indicated: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

these experiments identify microbe-responsive unconventional T cells as a rapid physiological source of GM-CSF, IFN-γ, and TNF-α and imply that the unique combination of cytokines secreted by unconventional T cells is key for the observed impact on neutrophils.

The particular requirement for TNF-α in the acquisition of the full APC phenotype is especially noteworthy when considering the cytokine/chemokine profiles in acutely infected patients. Plasma proteins that were highly elevated in sepsis patients included TNF-α as well as IL-6 and CXCL8 (Fig. 4E). Of note, there was a trend toward higher levels of TNF-α in patients with HMB-PP–positive infections (p = 0.09; data not shown). A proportion of individuals with sepsis also had increased plasma levels of GM-CSF, IFN-γ, and IL-1β, although this was not significant across the whole cohort (Fig. 4E). These findings confirm that the blood of sepsis patients contains proinflammatory mediators implicated in driving survival and activation of neutrophils, including their differentiation into APCs.

Unconventional T cell–primed neutrophils readily take up soluble Ags

We next tested the capacity of APC-like neutrophils to take up soluble Ags. Although freshly isolated neutrophils were not very efficient at endocytosing FITC-labeled BSA and dextran (10,000 Da) as model compounds, short-term exposure to Vγ9/Vδ2 T cell–conditioned medium led to a greatly enhanced uptake (Fig. 5A). With unconventional T cell–primed neutrophils kept in culture for 24 h before addition of BSA-FITC, Ag endocytosis was confined

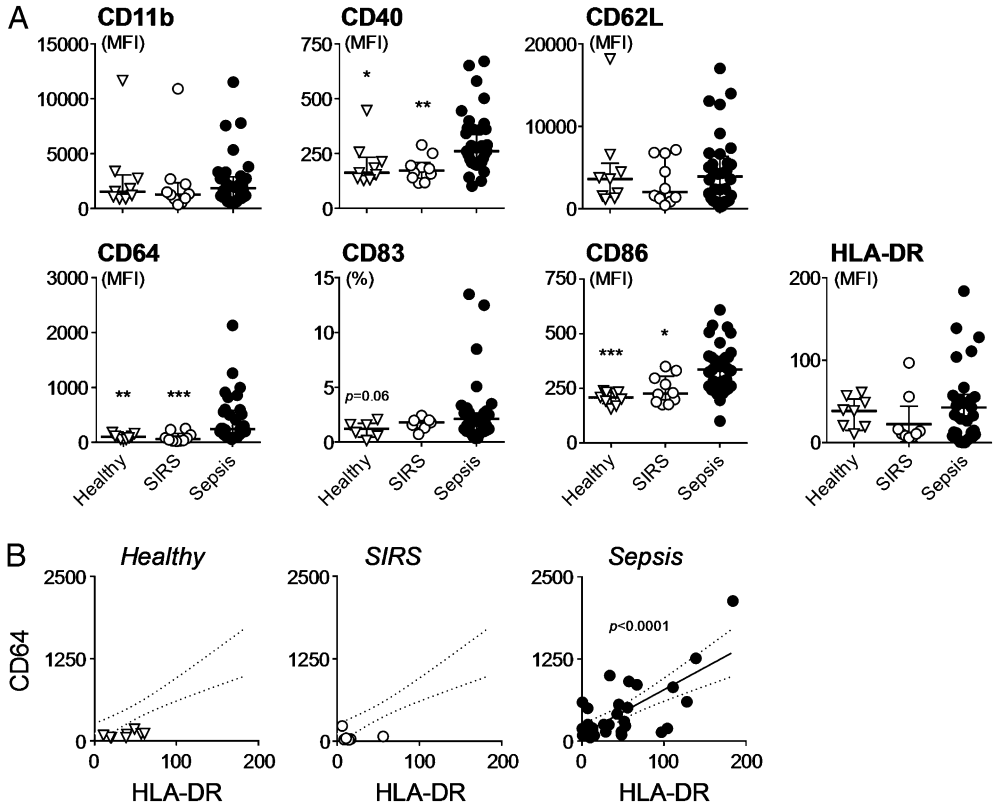

FIGURE 3. APC-like phenotype of circulating neutrophils during acute sepsis. (**A**) Surface expression of the indicated markers on circulating neutrophils in patients with SIRS (n = 14) or sepsis (n = 37) and in healthy controls (n = 10). Each data point represents an individual; lines and error bars depict medians and interquartile ranges. Data were analyzed using Kruskal–Wallis tests and Dunn's multiple comparison tests; comparisons were made with sepsis patients. (**B**) Correlation between surface expression of CD64 and HLA-DR on circulating neutrophils in healthy controls and in patients with SIRS or sepsis. Lines depict linear regression and 95% confidence bands as calculated for sepsis neutrophils. Differences were considered significant as indicated: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

to the $CD16^{high}$ APC-like population, whereas no such uptake was seen in the apoptotic $CD16^{low}$ population (Fig. 5B). In contrast, neutrophils cultured in medium alone showed no specific uptake of BSA in the $CD16^{high}$ population. These data indicate that unconventional T cells promote the uptake of exogenous Ags as a prerequisite for Ag processing and presentation by neutrophils.

Unconventional T cell–primed neutrophils are efficient APCs for $CD4^+$ and $CD8^+$ T cells

The functionality of cell surface–expressed HLA-DR on activated neutrophils was confirmed using the *S. aureus* superantigen, TSST-1, which cross-links MHC class II molecules with the TCR of $CD4^+$ T cells expressing a Vβ2 chain (35). Neutrophils exposed to Vγ9/Vδ2 T cell–conditioned medium or to a combination of GM-CSF, IFN-γ, and TNF-α were both capable of presenting TSST-1 to autologous $V\beta2^+$ $CD4^+$ T cells (Fig. 5C). When using the complex *M. tuberculosis* Ag, PPD, which requires intracellular processing, unconventional T cell–primed neutrophils displayed a striking capacity to trigger proliferation of both $CD4^+$ and $CD8^+$ T cells (Fig. 5D). Sequestration of TNF-α during the neutrophil-priming period by addition of sTNFR diminished both $CD4^+$ and $CD8^+$ T cell responses (Fig. 5E) as further confirmation of the key role for unconventional T cell–derived TNF-α in the acquisition of APC features by neutrophils.

Unconventional T cell–primed neutrophils cross-present Ags to $CD8^+$ T cells

Following up from the striking induction of PPD-specific $CD4^+$ and $CD8^+$ T cell responses, we assessed the potential of APC-like neutrophils to trigger $CD8^+$ T cell responses, by taking advantage of HLA-A2–restricted responder T cell lines specific for M1(p58–66), the immunodominant epitope of the influenza M1 protein (36–38). Using the M1(p58–66) peptide, which can be pulsed readily onto cell surface–associated MHC class I molecules for direct presentation to $CD8^+$ T cells, unconventional T cell–primed neutrophils showed a significantly improved Ag presentation, compared with freshly isolated neutrophils (Fig. 6A) and in agreement with the elevated levels of MHC class I molecules on APC-like neutrophils. Importantly, only unconventional T cell–primed neutrophils, but not freshly isolated neutrophils, were also able to induce robust responses by M1(p58–66)-specific responder $CD8^+$ T cells when utilizing the full-length M1 protein (Fig. 6A), a 251-aa–long Ag that requires uptake, processing, and loading of M1(p58–66) onto intracellular MHC class I molecules for cross-presentation to $CD8^+$ T cells (36–38). Control experiments supported the need for Ag uptake and processing, as recombinant M1 protein could not be pulsed directly onto neutrophils, demonstrating the absence of potential degradation products in the M1 protein preparation that might be able to bind directly to cell surface–associated MHC class I molecules on neutrophils or $CD8^+$ T cells (Fig. 6B). Neutrophils cultured for 48 h in the presence of GM-CSF and IFN-γ were also capable of enhanced presentation of M1(p58–66) peptide to M1-specific $CD8^+$ T cells. However, only neutrophils generated by incubation with a combination of GM-CSF, IFN-γ, and TNF-α readily processed the full-length M1 protein (Fig. 6A), demonstrating that TNF-α plays a pivotal role in the acquisition of a fully competent APC phenotype and function by neutrophils.

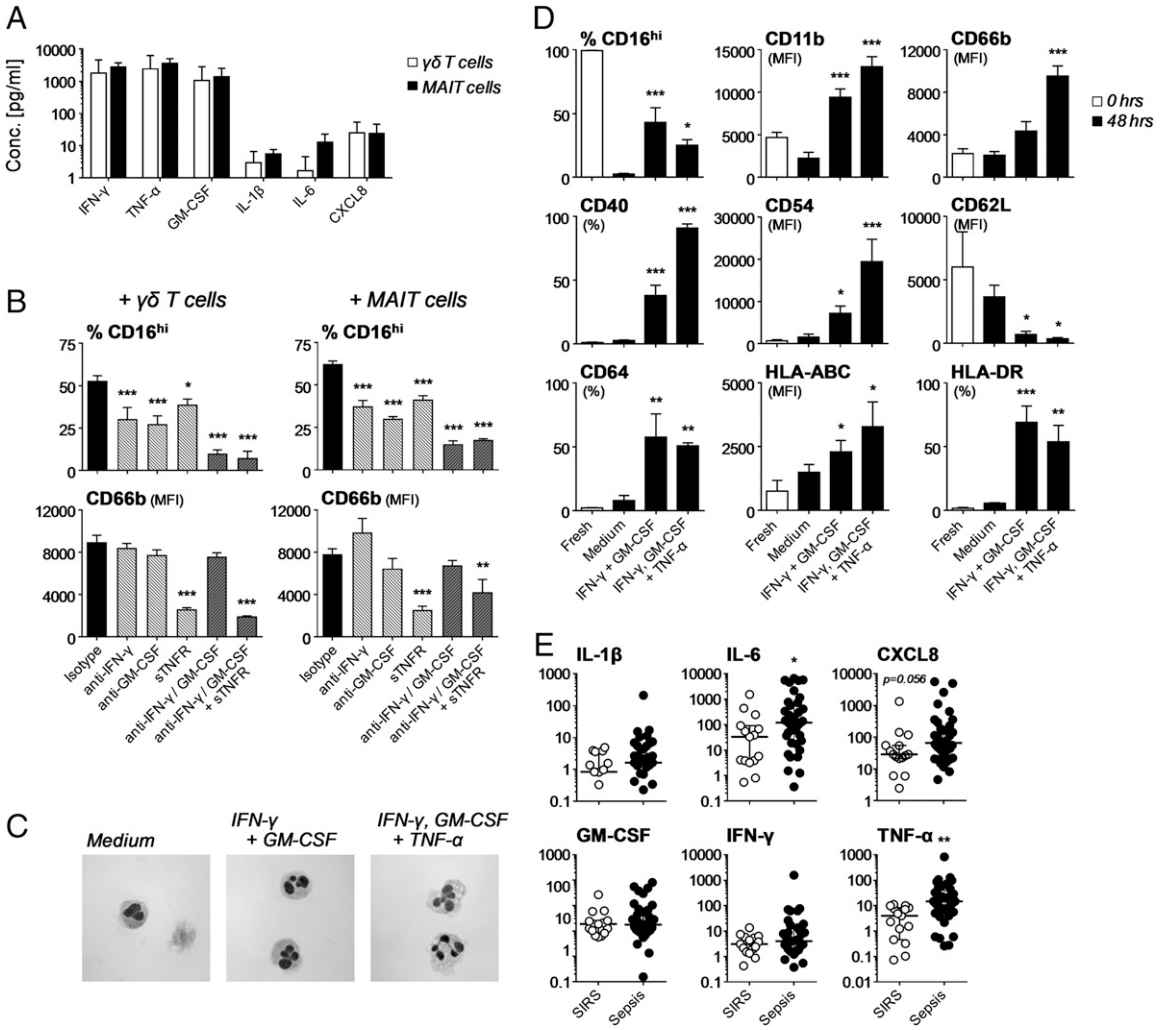

FIGURE 4. Effect of unconventional T cell–derived cytokines on neutrophil survival and APC marker expression. (**A**) Secretion of the indicated mediators into the culture supernatant by FACS-sorted Vγ9/Vδ2 T cells or MAIT cells stimulated overnight in the presence of HMB-PP or anti-CD3/CD28 beads, respectively, as detected using multiplex ELISA (means + SD, n = 2–3). (**B**) Neutrophil survival (as proportion of $CD16^{high}$ cells) and CD66b expression on $CD16^{high}$ neutrophils after 48-h culture in the presence of HMB-PP–activated Vγ9/Vδ2 T cell– or anti-CD3/CD28 MAIT cell–conditioned medium and neutralizing agents against GM-CSF, IFN-γ, and/or TNF-α (means + SD, n = 3). Data were analyzed by one-way ANOVA with Bonferroni's post hoc tests; comparisons were made with isotypes. (**C**) Morphological analysis of surviving neutrophils after 48-h culture in the absence or presence of GM-CSF, IFN-γ, and/or TNF-α (representative of two donors). Original magnification ×400. (**D**) Neutrophil survival and expression of the indicated markers on $CD16^{high}$ neutrophils after 48-h culture in the absence or presence of recombinant GM-CSF, IFN-γ, and/or TNF-α (means + SD, n = 3). Data were analyzed by one-way ANOVA with Bonferroni's post hoc tests; comparisons were made with medium controls. (**E**) Plasma levels of IL-1β, IL-6, CXCL8, GM-CSF, IFN-γ, and TNF-α in SIRS and sepsis patients (in pg/ml). Each data point represents an individual; lines and error bars depict medians and interquartile ranges. Differences between the two groups were analyzed using Mann–Whitney tests. Differences were considered significant as indicated: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Circulating neutrophils from sepsis patients are capable of cross-presenting Ags to $CD8^+$ T cells

It has not yet been established whether neutrophils are capable of triggering Ag-specific T cell responses in vivo. To translate our findings on APC-like neutrophils to the situation in acute infections, we isolated untouched neutrophils from sepsis patients to purities of 99.2–99.8%. Our experiments show that sepsis neutrophils and control neutrophils had a similar capacity to activate M1-specific responder $CD8^+$ T cells when pulsed with the peptide itself (Fig. 6C). Strikingly, only sepsis neutrophils, but not control neutrophils, were also able to take up the full-length M1 protein and cross-present the M1(p58–66) peptide to responder $CD8^+$ T cells (Fig. 6C, 6D), consistent with the differences in APC marker expression between patients and healthy individuals. These findings indicate that in acute sepsis neutrophils acquire an APC-like phenotype with the capacity to induce Ag-specific $CD8^+$ T cell responses that is reminiscent of neutrophils primed by unconventional T cells (Fig. 7).

Discussion

To our knowledge, the present study is the first demonstration that human neutrophils can assume Ag cross-presenting properties. Although our work does not formally demonstrate a causal link for the interaction of unconventional T cells and neutrophils in vivo, it does suggest a plausible scenario for the generation of APC-like neutrophils during acute infection. Our data support a model in which different types of unconventional T cells respond rapidly to neutrophils after phagocytosis of a broad range of bacteria at the site of infection, and in turn mediate the local differentiation of bystander neutrophils into APCs for both $CD4^+$ and $CD8^+$ T cells (Fig. 7). APC-like neutrophils may be particularly relevant for local responses by tissue-resident memory and/or freshly recruited

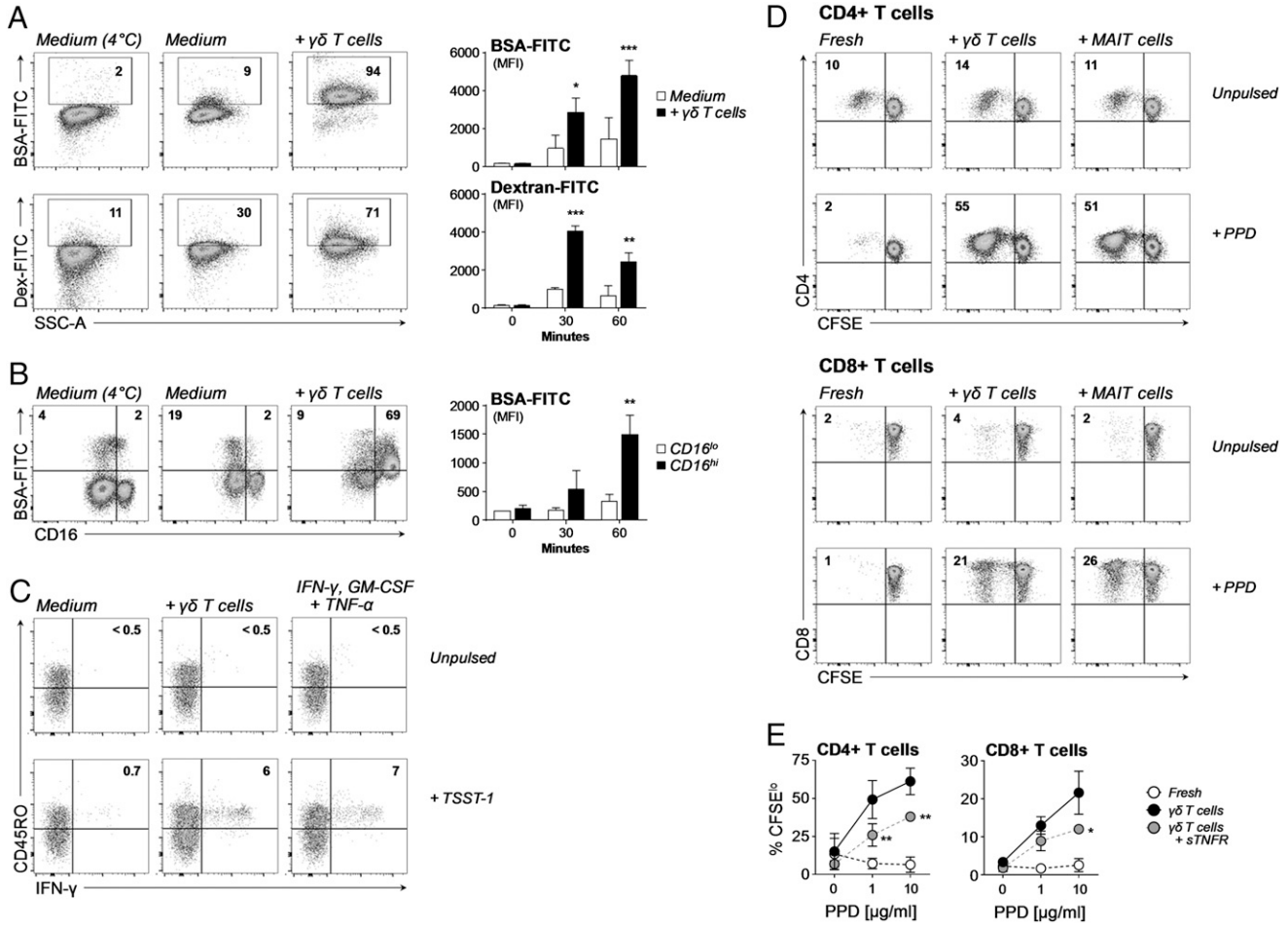

FIGURE 5. Efficient endocytosis of exogenous molecules and presentation of microbial Ags by unconventional T cell–primed neutrophils. (**A**) Endocytosis of FITC-labeled BSA and 10-kDa dextran by freshly isolated neutrophils incubated for 60 min at 4°C or at 37°C in the absence or presence of HMB-PP–activated γδ T cell supernatant. FACS plots are representative of two to three donors; specific uptake of FITC-labeled BSA and dextran by freshly isolated neutrophils was determined over 30 and 60 min (means + SD, $n = 2–3$). (**B**) Endocytosis of FITC-labeled BSA over 60 min by neutrophils that had been cultured overnight in the absence or presence of HMB-PP–activated γδ T cell supernatant. FACS plots are representative of three healthy donors; specific uptake of FITC-labeled BSA by γδ T cell–primed neutrophils was determined over 30 and 60 min (means + SD, $n = 2–3$). Data were analyzed by two-way ANOVA with Bonferroni's post hoc tests; comparisons were made with (A) medium controls or (B) $CD16^{low}$ cells. (**C**) IFN-γ production by superantigen-specific $CD4^+$ $V\beta2^+$ T cells in response to autologous neutrophils cultured for 48 h in medium or in the absence or presence of HMB-PP–activated Vγ9/Vδ2 T cell–conditioned medium or a combination of IFN-γ, GM-CSF, and TNF-α prior to pulsing with 10 ng/ml TSST-1 (representative of two donors). (**D**) Proliferation of $CD4^+$ and $CD8^+$ T cells in response to freshly isolated neutrophils and neutrophils cultured for 48 h in the presence of HMB-PP–activated Vγ9/Vδ2 T cell– or anti-CD3/CD28–activated MAIT cell–conditioned medium. Neutrophils were pulsed with 10 μg/ml PPD for 18 h prior to addition of CFSE-labeled bulk $CD3^+$ T cells; CFSE dilution of responder T cells was assessed after 7 d of coculture (representative of three donors). (**E**) Proliferation of CFSE-labeled $CD4^+$ and $CD8^+$ T cells in response to PPD-pulsed freshly isolated neutrophils and neutrophils cultured for 48 h in the presence of HMB-PP–activated Vγ9/Vδ2 T cell–conditioned medium with and without sTNFR. CFSE dilution of responder T cells was assessed after 7 d of coculture (means + SD, $n = 3$). Data were analyzed by one-way ANOVA with Bonferroni's post hoc tests; comparisons were made with Vγ9/Vδ2 T cell + sTNFR–treated neutrophils. Differences were considered significant as indicated: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

effector $CD4^+$ and $CD8^+$ T cells at the site of infection, rather than the priming of naive $CD4^+$ and $CD8^+$ T cells in secondary lymphoid tissues. Expression of the lymph node homing receptor CCR7 by activated neutrophils was reported before (30) but could not be confirmed in the current study (data not shown). Still, APC-like neutrophils may also gain access to inflamed draining lymph nodes through the action of inflammatory chemokines (7–10). Irrespective of the anatomical context, APC-like neutrophils may contribute to protective immune responses, by fighting the "first hit" infection as a result of inducing Ag-specific $CD4^+$ and $CD8^+$ T cells and by harnessing the T cell compartment against potential "second hit" infections. However, it is also thinkable that such an early induction of cytotoxic $CD8^+$ T cells may add to the systemic inflammatory response and ultimately lead to tissue damage and organ failure. Whereas the generation of APC-like neutrophils is likely to occur locally in the context of infected tissues, in severe inflammatory conditions, including sepsis, such APC-like neutrophils may eventually leak into the circulation and become detectable in blood. Larger stratified approaches are clearly needed to define the role of APC-like neutrophils in different infectious scenarios, locally and systemically, in clinically and microbiologically well-defined patient subgroups.

The presence of cross-presenting neutrophils in patients with sepsis is intriguing and may point to an essential role of APC-like neutrophils in acute disease. Sepsis patients who survive the primary infection often show signs of reduced surface expression of HLA-DR on monocytes and a relative tolerance of monocytes to LPS stimulation (47). As consequence of what is generally perceived as a loss of immune function, many patients are susceptible to subsequent nosocomial infections, including reactivation of

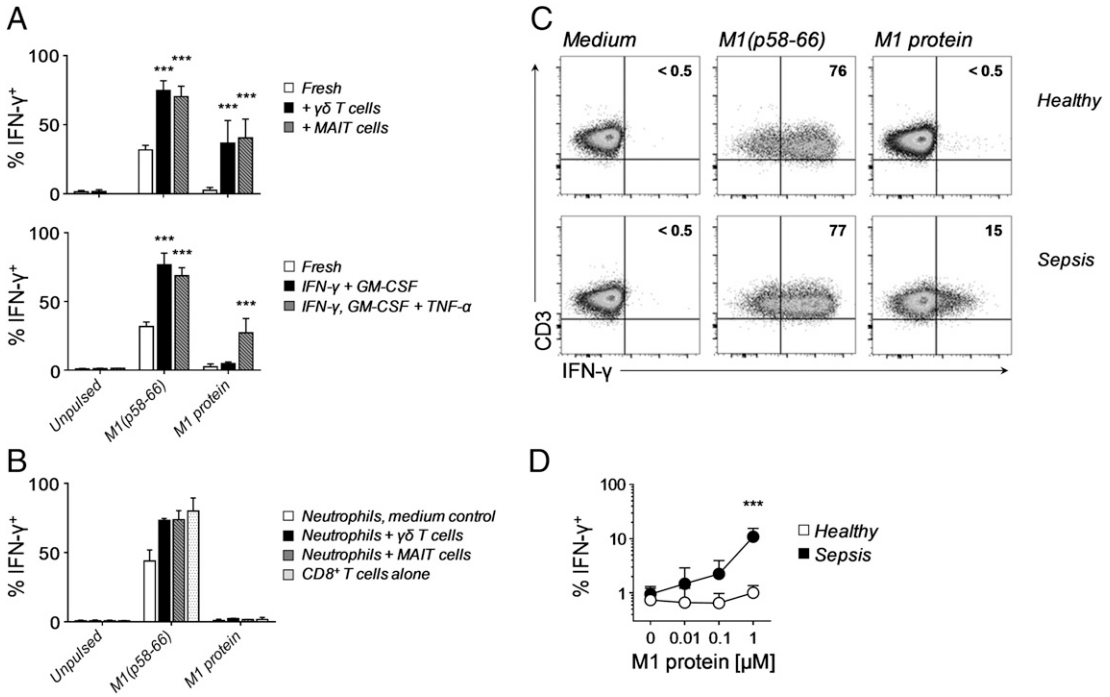

FIGURE 6. Cross-presentation of exogenous Ags by unconventional T cell–primed neutrophils and sepsis neutrophils. (**A**) IFN-γ production by Ag-specific $CD8^+$ T cells in response to neutrophils cultured for 48 h in the presence of HMB-PP–activated Vγ9/Vδ2 T cell– or anti-CD3/CD28–activated MAIT cell–conditioned medium (*top*), or neutrophils cultured for 48 h with the recombinant cytokines indicated (*bottom*). Neutrophils were pulsed for 1 h with 0.1 μM influenza M1(p58–66) peptide or for 18 h with recombinant M1 protein (means + SD, $n = 3$). Data were analyzed by two-way ANOVA with Bonferroni's post hoc tests; comparisons were made with freshly isolated neutrophils. (**B**) Failure of M1 protein to be pulsed directly onto neutrophils, as judged by IFN-γ production of Ag-specific $CD8^+$ T cells alone or in response to neutrophils cultured for 48 h in the absence or presence of HMB-PP–activated Vγ9/Vδ2 T cell– or anti-CD3/CD28–activated MAIT cell–conditioned medium. Neutrophils were pulsed for 1 h with either 0.1 μM influenza M1 (p58–66) peptide or 1 μM recombinant M1 protein; $CD8^+$ T cells were incubated directly with the peptide or M1 protein (means + SD, $n = 2$). (**C**) IFN-γ production by M1-specific $CD8^+$ T cells in response to freshly isolated neutrophils loaded with 0.1 μM synthetic M1(p58–66) peptide or 1 μM M1 protein. Data shown are representative of three $HLA\text{-}A2^+$ sepsis patients and three $HLA\text{-}A2^+$ healthy volunteers as controls. Sepsis patients recruited for these APC assays had confirmed infections as identified by positive culture results: *Escherichia coli* (urine), *Klebsiella pneumoniae* (respiratory culture), and *Staphylococcus epidermidis* (blood), respectively. (**D**) Summary of all stimulation assays conducted, shown as percentage of IFN-γ–positive $CD8^+$ T cells in response to freshly isolated neutrophils loaded with peptide or the indicated concentrations of M1 protein (means ± SD, $n = 3$). Data were analyzed by two-way ANOVA with Bonferroni's post hoc tests. Differences were considered significant as indicated: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

latent viruses that are associated with high mortality rates (48, 49). Trials specifically targeted at reversing this apparent monocyte deactivation have shown promising clinical results (50). However, our present findings suggest that HLA-DR expression by circulating monocytes is a poor surrogate marker for a systemic immune suppression and rather indicate that, contrary to the proposed general loss of function, certain cells such as neutrophils may actually assume APC properties under such conditions, as evidence of a gain of new function. Yet, with a complex and multilayered clinical phenomenon such as sepsis it is challenging to dissect the relevance of APC-like neutrophils for infection resolution and clinical outcome in vivo.

With their unique ability to recognize microbial metabolites in a non-MHC–restricted manner, unconventional T cells such as Vγ9/Vδ2 T cells and MAIT cells greatly outnumber Ag-specific conventional $CD4^+$ and $CD8^+$ T cells at the site of infection and represent early and abundant sources of proinflammatory cytokines (23, 27), among which GM-CSF, IFN-γ, and TNF-α each make key contributions. Although conventional T cells may produce a similar combination of cytokines and provide similar signals to neutrophils, preliminary findings in our laboratory indicate that Vγ9/Vδ2 T cells and MAIT cells represent together up to 50% of all TNF-α–producing T cells among peritoneal cells stimulated with bacterial extracts, suggesting that these two cell types are indeed major producers of proinflammatory cytokines in response to microbial stimulation (A. Liuzzi and M. Eberl, unpublished observations). Although we cannot rule out a further contribution of contact-dependent mechanisms, this observation builds upon earlier studies describing the generation of human neutrophils expressing MHC class II through the action of recombinant cytokines in vitro (22, 51–56) and in vivo (57–59). Previous investigations reported an upregulation of MHC class II on activated neutrophils under the control of GM-CSF and IFN-γ, albeit the physiological source of those mediators during acute infection was not defined. Most importantly, in this study, we describe a direct role for TNF-α in the efficient induction of MHC class I–restricted $CD8^+$ T cell responses by neutrophils. Of note, plasma from sepsis patients was previously shown to induce some (upregulation of CD64), but not other features (upregulation of CD11b, loss of CD62L) (60) that are characteristic for unconventional T cell–primed neutrophils, indicating that circulating cytokines alone do not confer APC properties. In support of local cell-mediated processes at the site of inflammation, our findings evoke earlier descriptions of APC-like neutrophils characterized by MHC class II expression in infectious and noninfectious inflammatory scenarios such as periodontitis (17) and tuberculous pleuritis (20), in which locally activated Vγ9/Vδ2 T cells were found (61–63). These associations lend further support to the existence of a peripheral immune surveillance network comprised of distinct types of unconventional T cells and their crosstalk with

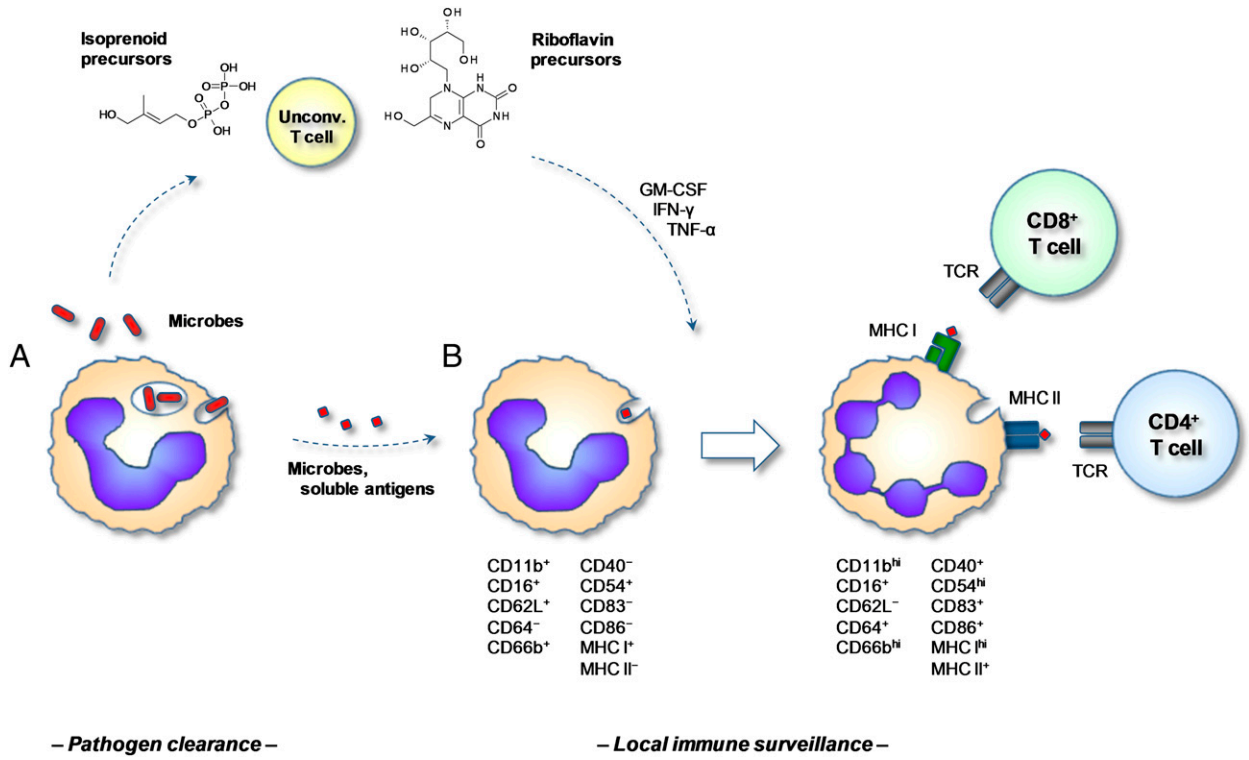

FIGURE 7. Proposed model for the local induction of APC-like neutrophils under the influence of microbe-responsive unconventional T cells. (**A**) Upon pathogen clearance, neutrophils release microbial metabolites into the microenvironment, where they stimulate local or freshly recruited unconventional T cells to release proinflammatory cytokines. (**B**) In the presence of unconventional T cell–derived mediators such as GM-CSF, IFN-γ, and TNF-α, bystander neutrophils acquire the capacity to act as APCs for tissue-resident and/or newly arriving effector and memory $CD4^+$ and $CD8^+$ T cells. Activated neutrophils may also gain access to inflamed draining lymph nodes and prime T cell responses in secondary lymphoid tissues (not depicted).

local immune and nonimmune cells. In the absence of unconventional T cell–derived signals, such as during sterile inflammation induced by LPS administration (64), neutrophils may not become fully primed, in accordance with our failure to induce APC-like neutrophils using LPS alone. Of note, a possible feedback regulation may require the activation of unconventional T cells to reach a certain threshold to overcome the inhibitory effect of bystander neutrophils (65–67).

Our present data demonstrate that both isoprenoid and riboflavin precursors are released by human neutrophils upon phagocytosis of live bacteria and depend on uptake by monocytes and loading onto butyrophilin 3A and MR1, respectively. The surprising similarities between Vγ9/Vδ2 T cells and MAIT cells illustrate their overlapping, yet distinct roles. Given the broad distribution of the nonmevalonate and riboflavin pathways across pathogenic, opportunistic, and commensal species, the vast majority of invading microbes is likely to be detected by either Vγ9/Vδ2 T cells or MAIT cells, or both. Our analysis of sepsis patients identified a systemic mobilization of Vγ9/Vδ2 T cells in response to HMB-PP–producing species, in support of their differential responsiveness to distinct groups of bacteria (28, 46). Because the present clinical study was conceived before information about the responsiveness of MAIT cells for riboflavin metabolites became available in the literature, we did not conduct a differential analysis for MAIT cells during acute sepsis. Of note, except for two cases of streptococcal infections, all bacterial and fungal pathogens identified in this patient cohort in fact possessed the riboflavin pathway, that is, were theoretically capable of stimulating MAIT cells. Intriguingly, Grimaldi et al. (68) recently reported a specific depletion of peripheral MAIT cells in sepsis patients with nonstreptococcal (i.e., riboflavin-producing) bacteria compared with infections caused by riboflavin-deficient species, which may indicate differences in the recruitment and retention of different types of unconventional T cells at sites of infection, depending on the nature of the causative pathogen and the underlying pathology (69–72). The contribution of tissue-resident and freshly recruited unconventional T cells to acute inflammatory responses has implications for clinical outcome and for the development of novel diagnostics and therapeutic interventions (46).

Taken together, our present study provides evidence 1) that Vγ9/Vδ2 T cells and MAIT cells respond similarly to microbial pathogens that produce the corresponding ligands when phagocytosed by human neutrophils, 2) that, once activated, both types of unconventional T cells trigger longer-term survival and differentiation of neutrophils into APC-like cells, 3) that unconventional T cell–primed neutrophils readily process exogenous Ags and prime both $CD4^+$ and $CD8^+$ T cells, and 4) that circulating neutrophils from patients with acute sepsis possess a similar APC-like phenotype and are capable of cross-presenting soluble proteins to Ag-specific $CD8^+$ T cells ex vivo. These findings define a possible physiological context for the generation of APC-like neutrophils in response to a broad range of microbial pathogens and imply a unique and decisive role for human unconventional T cells in orchestrating local inflammatory events and in shaping the transition of the innate to the adaptive phase of the antimicrobial immune response, with implications for diagnosis, therapy, and vaccination.

Acknowledgments

We are grateful to all patients and volunteers for participating in this study and we thank the clinicians and nurses for cooperation. We also thank Mark Toleman for clinical pathogens, Hassan Jomaa and Boris Illarionov for HMB-PP and DMRL, Andrew Thomas for recombinant M1 protein and

HLA-A2 tetramers, Ted Hansen and Daniel Olive for mAbs, Ann Kift-Morgan for multiplex ELISA measurements, Catherine Naseriyan for cell sorting, Chia-Te Liao for help with cytospins, and Marco Cassatella, Adrian Hayday, Ian Sabroe, and Phil Taylor for stimulating discussion.

Disclosures

The authors have no financial conflicts of interest.

References

1. Nathan, C. 2006. Neutrophils and immunity: challenges and opportunities. *Nat. Rev. Immunol.* 6: 173–182.
2. Mantovani, A., M. A. Cassatella, C. Costantini, and S. Jaillon. 2011. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat. Rev. Immunol.* 11: 519–531.
3. Mócsai, A. 2013. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J. Exp. Med.* 210: 1283–1299.
4. Müller, I., M. Munder, P. Kropf, and G. M. Hänsch. 2009. Polymorphonuclear neutrophils and T lymphocytes: strange bedfellows or brothers in arms? *Trends Immunol.* 30: 522–530.
5. Pillay, J., T. Tak, V. M. Kamp, and L. Koenderman. 2013. Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. *Cell. Mol. Life Sci.* 70: 3813–3827.
6. Cerutti, A., I. Puga, and G. Magri. 2013. The B cell helper side of neutrophils. *J. Leukoc. Biol.* 94: 677–682.
7. Kesteman, N., G. Vansanten, B. Pajak, S. M. Goyert, and M. Moser. 2008. Injection of lipopolysaccharide induces the migration of splenic neutrophils to the T cell area of the white pulp: role of CD14 and CXC chemokines. *J. Leukoc. Biol.* 83: 640–647.
8. Puga, I., M. Cols, C. M. Barra, B. He, L. Cassis, M. Gentile, L. Comerma, A. Chorny, M. Shan, W. Xu, et al. 2012. B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. *Nat. Immunol.* 13: 170–180.
9. Abadie, V., E. Badell, P. Douillard, D. Ensergueix, P. J. Leenen, M. Tanguy, L. Fiette, S. Saeland, B. Gicquel, and N. Winter. 2005. Neutrophils rapidly migrate via lymphatics after *Mycobacterium bovis* BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes. *Blood* 106: 1843–1850.
10. Chtanova, T., M. Schaeffer, S. J. Han, G. G. van Dooren, M. Nollmann, P. Herzmark, S. W. Chan, H. Satija, K. Camfield, H. Aaron, et al. 2008. Dynamics of neutrophil migration in lymph nodes during infection. *Immunity* 29: 487–496.
11. Beauvillain, C., Y. Delneste, M. Scotet, A. Peres, H. Gascan, P. Guermonprez, V. Barnaba, and P. Jeannin. 2007. Neutrophils efficiently cross-prime naive T cells in vivo. *Blood* 110: 2965–2973.
12. Abi Abdallah, D. S., C. E. Egan, B. A. Butcher, and E. Y. Denkers. 2011. Mouse neutrophils are professional antigen-presenting cells programmed to instruct Th1 and Th17 T-cell differentiation. *Int. Immunol.* 23: 317–326.
13. Ostanin, D. V., E. Kurmaeva, K. Furr, R. Bao, J. Hoffman, S. Berney, and M. B. Grisham. 2012. Acquisition of antigen-presenting functions by neutrophils isolated from mice with chronic colitis. *J. Immunol.* 188: 1491–1502.
14. Matsushima, H., S. Geng, R. Lu, T. Okamoto, Y. Yao, N. Mayuzumi, P. F. Kotol, B. J. Chojnacki, T. Miyazaki, R. L. Gallo, and A. Takashima. 2013. Neutrophil differentiation into a unique hybrid population exhibiting dual phenotype and functionality of neutrophils and dendritic cells. *Blood* 121: 1677–1689.
15. Geng, S., H. Matsushima, T. Okamoto, Y. Yao, R. Lu, K. Page, R. M. Blumenthal, N. L. Ward, T. Miyazaki, and A. Takashima. 2013. Emergence, origin, and function of neutrophil-dendritic cell hybrids in experimentally induced inflammatory lesions in mice. *Blood* 121: 1690–1700.
16. Iking-Konert, C., S. Vogt, M. Radsak, C. Wagner, G. M. Hänsch, and K. Andrassy. 2001. Polymorphonuclear neutrophils in Wegener's granulomatosis acquire characteristics of antigen presenting cells. *Kidney Int.* 60: 2247–2262.
17. Bisson-Boutelliez, C., N. Miller, D. Demarch, and M. C. Bene. 2001. CD9 and HLA-DR expression by crevicular epithelial cells and polymorphonuclear neutrophils in periodontal disease. *J. Clin. Periodontol.* 28: 650–656.
18. Cross, A., R. C. Bucknall, M. A. Cassatella, S. W. Edwards, and R. J. Moots. 2003. Synovial fluid neutrophils transcribe and express class II major histocompatibility complex molecules in rheumatoid arthritis. *Arthritis Rheum.* 48: 2796–2806.
19. Iking-Konert, C., B. Ostendorf, O. Sander, M. Jost, C. Wagner, L. Joosten, M. Schneider, and G. M. Hänsch. 2005. Transdifferentiation of polymorphonuclear neutrophils to dendritic-like cells at the site of inflammation in rheumatoid arthritis: evidence for activation by T cells. *Ann. Rheum. Dis.* 64: 1436–1442.
20. Alemán, M., S. S. de la Barrera, P. L. Schierloh, L. Alves, N. Yokobori, M. Baldini, E. Abbate, and M. C. Sasiain. 2005. In tuberculous pleural effusions, activated neutrophils undergo apoptosis and acquire a dendritic cell-like phenotype. *J. Infect. Dis.* 192: 399–409.
21. Sandilands, G. P., J. McCrae, K. Hill, M. Perry, and D. Baxter. 2006. Major histocompatibility complex class II (DR) antigen and costimulatory molecules on in vitro and in vivo activated human polymorphonuclear neutrophils. *Immunology* 119: 562–571.
22. Wagner, C., C. Iking-Konert, F. Hug, S. Stegmaier, V. Heppert, A. Wentzensen, and G. M. Hänsch. 2006. Cellular inflammatory response to persistent localized *Staphylococcus aureus* infection: phenotypical and functional characterization of polymorphonuclear neutrophils (PMN). *Clin. Exp. Immunol.* 143: 70–77.
23. Gold, M. C., and D. M. Lewinsohn. 2013. Co-dependents: MR1-restricted MAIT cells and their antimicrobial function. *Nat. Rev. Microbiol.* 11: 14–19.
24. Brennan, P. J., M. Brigl, and M. B. Brenner. 2013. Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. *Nat. Rev. Immunol.* 13: 101–117.
25. Vantourout, P., and A. Hayday. 2013. Six-of-the-best: unique contributions of γδ T cells to immunology. *Nat. Rev. Immunol.* 13: 88–100.
26. Bonneville, M., R. L. O'Brien, and W. K. Born. 2010. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat. Rev. Immunol.* 10: 467–478.
27. Eberl, M., and B. Moser. 2009. Monocytes and gammadelta T cells: close encounters in microbial infection. *Trends Immunol.* 30: 562–568.
28. Davey, M. S., C. Y. Lin, G. W. Roberts, S. Heuston, A. C. Brown, J. A. Chess, M. A. Toleman, C. G. Gahan, C. Hill, T. Parish, et al. 2011. Human neutrophil clearance of bacterial pathogens triggers anti-microbial γδ T cell responses in early infection. *PLoS Pathog.* 7: e1002040.
29. Welton, J. L., M. P. Morgan, S. Martí, M. D. Stone, B. Moser, A. K. Sewell, J. Turton, and M. Eberl. 2013. Monocytes and γδ T cells control the acute-phase response to intravenous zoledronate: insights from a phase IV safety trial. *J. Bone Miner. Res.* 28: 464–471.
30. Beauvillain, C., P. Cunin, A. Doni, M. Scotet, S. Jaillon, M. L. Loiry, G. Magistrelli, K. Masternak, A. Chevailler, Y. Delneste, and P. Jeannin. 2011. CCR7 is involved in the migration of neutrophils to lymph nodes. *Blood* 117: 1196–1204.
31. Kastenmüller, W., P. Torabi-Parizi, N. Subramanian, T. Lämmermann, and R. N. Germain. 2012. A spatially-organized multicellular innate immune response in lymph nodes limits systemic pathogen spread. *Cell* 150: 1235–1248.
32. Barral, P., M. D. Sánchez-Niño, N. van Rooijen, V. Cerundolo, and F. D. Batista. 2012. The location of splenic NKT cells favours their rapid activation by blood-borne antigen. *EMBO J.* 31: 2378–2390.
33. Davey, M. S., N. Tamassia, M. Rossato, F. Bazzoni, F. Calzetti, K. Bruderek, M. Sironi, L. Zimmer, B. Bottazzi, A. Mantovani, et al. 2011. Failure to detect production of IL-10 by activated human neutrophils. *Nat. Immunol.* 12: 1017–1018, author reply 1018–1020.
34. Brandes, M., K. Willimann, and B. Moser. 2005. Professional antigen-presentation function by human gammadelta T cells. *Science* 309: 264–268.
35. Eberl, M., G. W. Roberts, S. Meuter, J. D. Williams, N. Topley, and B. Moser. 2009. A rapid crosstalk of human gammadelta T cells and monocytes drives the acute inflammation in bacterial infections. *PLoS Pathog.* 5: e1000308.
36. Brandes, M., K. Willimann, G. Bioley, N. Lévy, M. Eberl, M. Luo, R. Tampé, F. Lévy, P. Romero, and B. Moser. 2009. Cross-presenting human gammadelta T cells induce robust CD8+ alphabeta T cell responses. *Proc. Natl. Acad. Sci. USA* 106: 2307–2312.
37. Meuter, S., M. Eberl, and B. Moser. 2010. Prolonged antigen survival and cytosolic export in cross-presenting human gammadelta T cells. *Proc. Natl. Acad. Sci. USA* 107: 8730–8735.
38. Khan, M. W. A., S. M. Curbishley, H.-C. Chen, A. D. Thomas, H. Pircher, D. Mavilio, N. M. Steven, M. Eberl, and B. Moser. 2014. Expanded human blood-derived γδ T cells display potent antigen-presentation functions. *Front. Immunol.* 5: 344.
39. Morita, C. T., C. Jin, G. Sarikonda, and H. Wang. 2007. Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vgamma2Vdelta2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. *Immunol. Rev.* 215: 59–76.
40. Le Bourhis, L., E. Martin, I. Péguillet, A. Guihot, N. Froux, M. Coré, E. Lévy, M. Dusseaux, V. Meyssonnier, V. Premel, et al. 2010. Antimicrobial activity of mucosal-associated invariant T cells. *Nat. Immunol.* 11: 701–708.
41. Kjer-Nielsen, L., O. Patel, A. J. Corbett, J. Le Nours, B. Meehan, L. Liu, M. Bhati, Z. Chen, L. Kostenko, R. Reantragoon, et al. 2012. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* 491: 717–723.
42. López-Sagaseta, J., C. L. Dulberger, A. McFedries, M. Cushman, A. Saghatelian, and E. J. Adams. 2013. MAIT recognition of a stimulatory bacterial antigen bound to MR1. *J. Immunol.* 191: 5268–5277.
43. Corbett, A. J., S. B. Eckle, R. W. Birkinshaw, L. Liu, O. Patel, J. Mahony, Z. Chen, R. Reantragoon, B. Meehan, H. Cao, et al. 2014. T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* 509: 361–365.
44. Harly, C., Y. Guillaume, S. Nedellec, C. M. Peigné, H. Mönkkönen, J. Mönkkönen, J. Li, J. Kuball, E. J. Adams, S. Netzer, et al. 2012. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T-cell subset. *Blood* 120: 2269–2279.
45. Sandstrom, A., C. M. Peigné, A. Léger, J. E. Crooks, F. Konczak, M. C. Gesnel, R. Breathnach, M. Bonneville, E. Scotet, and E. J. Adams. 2014. The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vγ9Vδ2 T cells. *Immunity* 40: 490–500.
46. Lin, C. Y., G. W. Roberts, A. Kift-Morgan, K. L. Donovan, N. Topley, and M. Eberl. 2013. Pathogen-specific local immune fingerprints diagnose bacterial infection in peritoneal dialysis patients. *J. Am. Soc. Nephrol.* 24: 2002–2009.
47. Hotchkiss, R. S., G. Monneret, and D. Payen. 2013. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat. Rev. Immunol.* 13: 862–874.
48. Angus, D. C., and T. van der Poll. 2013. Severe sepsis and septic shock. *N. Engl. J. Med.* 369: 840–851.
49. Osawa, R., and N. Singh. 2009. Cytomegalovirus infection in critically ill patients: a systematic review. *Crit. Care* 13: R68.
50. Meisel, C., J. C. Schefold, R. Pschowski, T. Baumann, K. Hetzger, J. Gregor, S. Weber-Carstens, D. Hasper, D. Keh, H. Zuckermann, et al. 2009. Granulocyte-

macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. *Am. J. Respir. Crit. Care Med.* 180: 640–648.
51. Gosselin, E. J., K. Wardwell, W. F. Rigby, and P. M. Guyre. 1993. Induction of MHC class II on human polymorphonuclear neutrophils by granulocyte/macrophage colony-stimulating factor, IFN-gamma, and IL-3. *J. Immunol.* 151: 1482–1490.
52. Smith, W. B., L. Guida, Q. Sun, E. I. Korpelainen, C. van den Heuvel, D. Gillis, C. M. Hawrylowicz, M. A. Vadas, and A. F. Lopez. 1995. Neutrophils activated by granulocyte-macrophage colony-stimulating factor express receptors for interleukin-3 which mediate class II expression. *Blood* 86: 3938–3944.
53. Fanger, N. A., C. Liu, P. M. Guyre, K. Wardwell, J. O'Neil, T. L. Guo, T. P. Christian, S. P. Mudzinski, and E. J. Gosselin. 1997. Activation of human T cells by major histocompatibility complex class II expressing neutrophils: proliferation in the presence of superantigen, but not tetanus toxoid. *Blood* 89: 4128–4135.
54. Oehler, L., O. Majdic, W. F. Pickl, J. Stöckl, E. Riedl, J. Drach, K. Rappersberger, K. Geissler, and W. Knapp. 1998. Neutrophil granulocyte-committed cells can be driven to acquire dendritic cell characteristics. *J. Exp. Med.* 187: 1019–1028.
55. Yamashiro, S., J. M. Wang, D. Yang, W. H. Gong, H. Kamohara, and T. Yoshimura. 2000. Expression of CCR6 and CD83 by cytokine-activated human neutrophils. *Blood* 96: 3958–3963.
56. Radsak, M., C. Iking-Konert, S. Stegmaier, K. Andrassy, and G. M. Hänsch. 2000. Polymorphonuclear neutrophils as accessory cells for T-cell activation: major histocompatibility complex class II restricted antigen-dependent induction of T-cell proliferation. *Immunology* 101: 521–530.
57. Mudzinski, S. P., T. P. Christian, T. L. Guo, E. Cirenza, K. R. Hazlett, and E. J. Gosselin. 1995. Expression of HLA-DR (major histocompatibility complex class II) on neutrophils from patients treated with granulocyte-macrophage colony-stimulating factor for mobilization of stem cells. *Blood* 86: 2452–2453.
58. Zarco, M. A., J. M. Ribera, N. Villamor, A. Balmes, A. Urbano Ispizua, and E. Feliu. 1998. Phenotypic changes in neutrophil granulocytes after G-CSF administration in patients with acute lymphoblastic leukemia under chemotherapy. *Haematologica* 83: 573–575.
59. Reinisch, W., C. Lichtenberger, G. Steger, W. Tillinger, O. Scheiner, A. Gangl, D. Maurer, and M. Willheim. 2003. Donor dependent, interferon-gamma induced HLA-DR expression on human neutrophils in vivo. *Clin. Exp. Immunol.* 133: 476–484.
60. Lewis, S. M., D. F. Treacher, L. Bergmeier, S. D. Brain, D. J. Chambers, J. D. Pearson, and K. A. Brown. 2009. Plasma from patients with sepsis upregulates the expression of CD49d and CD64 on blood neutrophils. *Am. J. Respir. Cell Mol. Biol.* 40: 724–732.
61. Yokobori, N., P. Schierloh, L. Geffner, L. Balboa, M. Romero, R. Musella, J. Castagnino, G. De Stéfano, M. Alemán, S. de la Barrera, et al. 2009. CD3 expression distinguishes two gammadeltaT cell receptor subsets with different phenotype and effector function in tuberculous pleurisy. *Clin. Exp. Immunol.* 157: 385–394.
62. Kawahara, K., M. Fukunaga, T. Takata, M. Kawamura, M. Morishita, and Y. Iwamoto. 1995. Immunohistochemical study of γ δ T cells in human gingival tissues. *J. Periodontol.* 66: 775–779.
63. Gemmell, E., and G. J. Seymour. 1995. γ δ T lymphocytes in human periodontal disease tissue. *J. Periodontol.* 66: 780–785.
64. Pillay, J., V. M. Kamp, E. van Hoffen, T. Visser, T. Tak, J. W. Lammers, L. H. Ulfman, L. P. Leenen, P. Pickkers, and L. Koenderman. 2012. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J. Clin. Invest.* 122: 327–336.
65. Wingender, G., M. Hiss, I. Engel, K. Peukert, K. Ley, H. Haller, M. Kronenberg, and S. von Vietinghoff. 2012. Neutrophilic granulocytes modulate invariant NKT cell function in mice and humans. *J. Immunol.* 188: 3000–3008.
66. Sabbione, F., M. L. Gabelloni, G. Ernst, M. S. Gori, G. Salamone, M. Oleastro, A. Trevani, J. Geffner, and C. C. Jancic. 2014. Neutrophils suppress γδ T-cell function. *Eur. J. Immunol.* 44: 819–830.
67. Kalyan, S., V. Chandrasekaran, E. S. Quabius, T. K. Lindhorst, and D. Kabelitz. 2014. Neutrophil uptake of nitrogen-bisphosphonates leads to the suppression of human peripheral blood γδ T cells. *Cell. Mol. Life Sci.* 71: 2335–2346.
68. Grimaldi, D., L. Le Bourhis, B. Sauneuf, A. Dechartres, C. Rousseau, F. Ouaaz, M. Milder, D. Louis, J. D. Chiche, J. P. Mira, et al. 2014. Specific MAIT cell behaviour among innate-like T lymphocytes in critically ill patients with severe infections. *Intensive Care Med.* 40: 192–201.
69. Matsushima, A., H. Ogura, K. Fujita, T. Koh, H. Tanaka, Y. Sumi, K. Yoshiya, H. Hosotsubo, Y. Kuwagata, T. Shimazu, and H. Sugimoto. 2004. Early activation of gammadelta T lymphocytes in patients with severe systemic inflammatory response syndrome. *Shock* 22: 11–15.
70. Venet, F., J. Bohé, A. L. Debard, J. Bienvenu, A. Lepape, and G. Monneret. 2005. Both percentage of gammadelta T lymphocytes and CD3 expression are reduced during septic shock. *Crit. Care Med.* 33: 2836–2840.
71. Andreu-Ballester, J. C., C. Tormo-Calandín, C. Garcia-Ballesteros, J. Pérez-Griera, V. Amigó, A. Almela-Quilis, J. Ruiz del Castillo, C. Peñarroja-Otero, and F. Ballester. 2013. Association of γδ T cells with disease severity and mortality in septic patients. *Clin. Vaccine Immunol.* 20: 738–746.
72. Heffernan, D. S., S. F. Monaghan, C. S. Chung, W. G. Cioffi, S. Gravenstein, and A. Ayala. 2014. A divergent response of innate regulatory T-cells to sepsis in humans: circulating invariant natural killer T-cells are preserved. *Hum. Immunol.* 75: 277–282.