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Human γδ T Cells: A Lymphoid Lineage Cell Capable of Professional Phagocytosis

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Professional phagocytosis in mammals is considered to be performed exclusively by myeloid cell types. In this study, we demonstrate, for the first time, that a mammalian lymphocyte subset can operate as a professional phagocyte. By using confocal microscopy, transmission electron microscopy, and functional Ag presentation assays, we find that freshly isolated human peripheral blood γδ T cells can phagocytose *Escherichia coli* and 1 μm synthetic beads via Ab opsonization and CD16 (FcγRIII), leading to Ag processing and presentation on MHC class II. In contrast, other CD16⁺ lymphocytes, i.e., CD16⁺/CD56⁺ NK cells, were not capable of such functions. These findings of distinct myeloid characteristics in γδ T cells strongly support the suggestion that γδ T cells are evolutionarily ancient lymphocytes and have implications for our understanding of their role in transitional immunity and the control of infectious diseases and cancer. The Journal of Immunology, 2009, 183: 5622–5629.

Immune cells express hard programmed receptors that sense molecular structures on microbes and altered self-cells which enable the uptake of these agents as a broad first line defense. Adaptive immune cells evolve clonally within an individual by somatic rearrangement of specific receptors providing a delayed but tailored response. Cross-talk between the two is central to an appropriate immune response (1, 2). Myeloid cells such as monocytes, macrophages, neutrophils, and myeloid dendritic cells (DCs)³ clearly display innate characteristics, while lymphoid lineage B and αβ T cells represent the classical adaptive response. γδ T cells, however, display characteristics of both. γδ T cells, while sharing αβ T cell functions, also perform immune surveillance of an innate character and are the only major set of tissue-resident T cells (3). Compared with αβ T cells, the γδ TCR repertoire is very restricted and matched to the respective tissue type these cells reside in (4). This restricted repertoire is directed against non-MHC-restricted Ags common to pathogens and stressed self-cells in a fashion reminiscent of pattern recognition receptors on innate myeloid cells. This is despite the fact that their potential TCR diversity actually exceeds αβ TCRs. Although they constitute only 0.5–10% of total T cells, γδ T cells expand rapidly upon direct encounter with ligands common to pathogens and cell stress markers without the need for conventional T cell activation mechanisms.

γδ T cells from several mammalian species can surprisingly present Ags to CD4⁺ αβ T cells on MHC class II (5–8). Brandes et al. have shown that activated human γδ T cells express MHC class II and can professionally present protein Ags to both naive CD4⁺ and CD8⁺ αβ T cells while αβ T cell controls could not (5, 9). In addition, it seems that activated γδ T cells up-regulate CCR7 and home to local lymph nodes where they interact with αβ T cells and other APCs (10, 11). The activation profile and behavior of γδ T cells bears an uncanny resemblance to that of the innate myeloid lineage DC. However, it remains unknown how γδ T cells acquire Ag (12).

Professional Ag presentation is a hallmark of innate immunity and is often associated with professional phagocytosis. Professional phagocytosis, as defined by phagocytosis by myeloid lineage monocytes, DCs, and neutrophils, is a tissue resident activity, which allows for sampling of the local environment as part of tissue homeostasis and in readiness for pathogens (13). It is an evolutionary development of unicellular phagocytosis, which in jawed vertebrates was the starting point for the processing and presentation of Ags to the adaptive immune system. Given that γδ T cells are hypothesized to predate the development of other lymphoid lineage B and αβ T cells (14), that they express the phagocytic Fc receptor for IgG, CD16 (FcγRIII) (15, 16), and their capacity as professional APCs (5, 9), we investigated their capacity for phagocytosis. We find in this study that human peripheral blood γδ T cells can phagocytose both 1-μm synthetic beads as well as *Escherichia coli* via CD16-mediated uptake, and moreover that this is functionally linked to Ag processing and presentation on MHC class II.

Materials and Methods

**Isolation of γδ T cells**

Peripheral venous blood from healthy donors was heparinized with 3 IU heparin/ml blood, diluted 1/1 with sterile PBS, and layered over 1/3 volume of Ficoll-Paque. Samples were centrifuged for 2200 rpm (Sorvall Leg-End Mach 1.6R) for 30 min at room temperature with no brake. A small volume of plasma was removed and saved for later to block Fc receptors.

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5. Abbreviations used in this paper: DC, dendritic cell; LCL, lymphoblastic cell line; TEM, transmission electron microscopy; M1, influenza matrix 1 protein; IPP, isopentenyl pyrophosphate; TMR, tetramethylrhodamine; mil-2, murine IL-2.

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during staining. The PBMC layer was removed and washed in 4°C PBS before magnetic selection. Fresh γδ T cells and NK cells were isolated from PBMCs using the anti-TCR γδ and CD56/CD16 MicroBead Kits (Miltenyi Biotec), respectively, to a purity of 95% (supplementary Fig. S1). All steps were performed on ice to minimize nonspecific staining. γδ T cell preparations were activated by coculture with HLA-DRB1*0701 lymphoblastic cell lines (LCLs), 100 IU/ml human IL-2, and 50 μM isopentenyl pyrophosphate, as previously described (5).

Incubation with tetramethylrhodamine E. coli and fluorescent red latex beads and staining for immunofluorescence

Four × 10^4 freshly isolated peripheral blood γδ T cells were incubated with 20 μl of rabbit IgG opsonised tetramethylrhodamine E. coli (Molecular Probes BioParticles, Inc.; E. coli E-2682 and Opsonizing Reagent E-2870, reconstituted according to the manufacturer’s instructions) in 100 μl of serum-free DMEM in 1 cm² square wells on polylysine coated slides for 45 min at 37°C. As a control for active phagocytosis, coverslips were incubated on ice and no phagocytosis was observed (data not shown). One micrometer fluorescent red latex beads (Sigma-Aldrich, t-2787) were passively coated with BSA and opsonized with rabbit anti-BSA IgG. Opsonized beads were incubated with γδ T cells as described above. After 45 min, slides were fixed with 4% formaldehyde, permeablized with 0.5% Triton, and blocked with 5% autologous plasma before staining. Slides were stained with mouse anti-human TCR γδ (BD Bioscience 555716) and goat anti-mouse Cy5 (Caltag M5011), AlexaFluor 488 phalloidin (Invitrogen A12379), and DAPI (Vectashield H-1500) before imaging on a laser scanning microscope system (TCS NT) (Leica).

Transmission electron microscopy (TEM)

After incubation with E. coli, γδ T cells were washed in PBS and fixed in 4% formaldehyde and 2.5% glutaraldehyde. Preparation for TEM was done following procedures described previously (17). In brief, after fixation γδ T cells were pelleted, transferred to glass slides, and allowed to dry on a hotplate at 37°C before embedding in 2% agarose solution. Agarose embedded blocks were then fixed in EM fixative (2.5% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer solution (pH 7.4)) at 4°C for 4 h. After 4 h, the blocks were washed three times with 0.1 M phosphate buffer solution before post fixing with 1% osmium tetroxide solution in wash buffer at 4°C overnight. Blocks were then dehydrated in graded ethanol, embedded in pure Epon, and kept at 60°C for 3 days. Ninety nanometer ultrathin sections were stained with 8% uranyl acetate and lead citrate before observation under electron microscope.

Generation of A1C5 hybridomas

All animal experiments were done in accordance with the guidelines laid out by the Institutional Animal Care and Use Committee at Case Western Reserve University. Generation of the T cell hybridoma, A1C5 was similar to previously published methods (18). In brief, mice transgenic for HLA-DRB1*0101 (Dennis Zaller, Merck Laboratories, New York, NY) were injected in the footpad with recombinant influenza matrix (M1) Ag purged of cytokines (5623 The Journal of Immunology) and fluorescent red latex beads. T cells were blocked with a mouse monoclonal IgG1 anti-CD16 block (BD Biosciences) for 30 min at 37°C before embedding in 2% agarose solution. Agarose embedded blocks were then fixed in EM fixative (2.5% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer solution (pH 7.4)) at 4°C for 4 h. After 4 h, the blocks were washed three times with 0.1 M phosphate buffer solution before post fixing with 1% osmium tetroxide solution in wash buffer at 4°C overnight. Blocks were then dehydrated in graded ethanol, embedded in pure Epon, and kept at 60°C for 3 days. Ninety nanometer ultrathin sections were stained with 8% uranyl acetate and lead citrate before observation under electron microscope.

Results

Confluent microscopic imaging of phagocytosis by γδ T cells

To assess their phagocytic capacity, γδ T cells were directly isolated from fresh blood by positive anti-TCR γδ selection on magnetic microbeads to a purity of 95% (supplementary Fig. S1). Cells were then pulsed with IgG opsonized tetramethylrhodamine (TMR)-labeled E. coli or IgG opsonized 1 μM fluorescent red latex beads and stained for immunofluorescence.

γδ T cells were identified by positive (Fig. 1A) and often punctate (Fig. 1B) anti-TCR γδ staining both on the surface and inside the cell. This punctate staining was consistent with TCR-CD3 clustering on activated γδ T cells (20) and reports of TCR γδ clustering on γδ T cells after TCR ligation (21), and was likely due to some activation during selection. γδ T cells were also delineated from the few contaminating monocytes by their distinct morphology. γδ T cells were <10 μm across, with round dense nuclei surrounded by a thin round ring of cytoplasm. Monocytes were >10 μm, with distinct horseshoe-shaped nuclei surrounded by

3 The online version of this article contains supplementary material.

4 Recombinant his-tagged M1 was conjugated to 1 μM polystyrene beads (DynaBeads TALON 101.01D) according to the manufacturer’s instructions. Beads were washed extensively to remove all unconjugated M1 protein from preparations (Fig. 4B) such that any MHC class II presentation of M1 was exclusively derived from phagocytosed M1 beads.

Presentation of M1-conjugated beads to A1C5 hybridomas

In brief, 10^5 γδ T cells were seeded in 100 μl of serum-free DMEM in triplicate wells of 96-well round-bottom tissue culture plates. Negative control wells with γδ T cells were seeded with 100 μl of serum-free DMEM. Wells then received PBS, nonopsonised M1 beads, or anti-M1 rabbit antisera (19) opsonized M1 beads and were incubated at 37°C/5% CO₂, h. After 1 h, 10^5 A1C5 hybridomas were added in 100 μl to each well. For activation, 50 μM IPP, 100 IU/ml recombinant human IL-2, and 10^5 DRB1*0701 LCLs were added to γδ T cell wells and negative control wells. Plates were then incubated at 37°C/5% CO₂ for 24–36 h. Murine IL-2 secretion by A1C5 hybridomas was assessed by ELISA for murine IL-2 (R&D Systems Mouse DuoSet DY402).

Inhibiting active phagocytosis and Ag processing with cytochalasin D and chloroquine

To inhibit Ag processing, γδ T cells were incubated with 50 μg/ml chloroquine, an inhibitor of phagosomal acidification, for one hour before pulsing. To block active phagocytosis, γδ T cells were incubated with 10 μg/ml cytochalasin D diluted in DMSO for one hour before pulsing. γδ T cells were also pulsed in the equivalent 1/1000 dilution of DMSO to control for any effect the drug solvent might have on the assay.

CD16 and MHC class II blocking

γδ T cells were blocked with a mouse monoclonal IgG1 anti-CD16 blocking Ab (clone LNK16, Abcam) or nonspecific mouse IgG1 at 10 μg/ml for 30 min at 37°C/5% CO₂ before pulsing with M1 beads. Likewise, γδ T cells were blocked with an azide-free mouse monoclonal IgG2a anti-HLA-DR (clone L243, BD Biosciences) or nonspecific mouse IgG2a (BD Biosciences) for 30 min before addition of responder T cells.

Controlling for PBMC contamination

In brief, 10^5 cells from the γδ T cell preparation were used for presentation of which <5000 (5%) were monocytes. To control for the possible contribution of monocytes and the very few peripheral blood DCs, 5 × 10^4 donor PBMCs were used as a source of at least 5000 monocytes (donor monocyte content ranged from 13–20%, data not shown) and were incubated with the same Ags and under the same activating conditions in parallel with the experiments described above. We also sorted γδ T cells from PBMCs on TCR-γδ expression by flow cytometry reducing monocyte contamination to <0.2% (Fig. 5Bii). Due to lower yields, we used 2.5 × 10^5 highly pure flow cytometry sorted γδ T cells as Ag presenters (Fig. 5Bii).
large irregular cytoplasms giving a high cytoplasm-to-nucleus ratio (Fig. 1C). The majority of γδ T cells observed displayed typical lymphocyte morphology but up to 10% had distinct plasma membrane dendritic processes resembling pseudopods formed by phagocytic cells (Fig. 1D). Otherwise, these cells were of the same size and had the same nuclear morphology and TCRγδ staining as other γδ T cells. When pulsed with IgG-opsonized TMR E. coli, these “hairy” γδ T cells had more E. coli adsorbed onto their surface than smooth γδ T cells (Fig. 1, E and F). We found up to 10% of γδ T cells with internalized E. coli (Fig. 1G). To confirm that the E. coli were indeed inside the cells, “stacks” of a minimum of nine images were acquired from different levels of the cell. Two examples of such cells are shown in supplemental Fig. S3 and S4, of which the stack in Fig. S3 corresponds to the cell displayed in Fig. 1G. Given recent reports of teleost fish and Xenopus dendritic-like B cells capable of phagocytosis (22), we speculate that the dendritic-like population of γδ T cells were responsible. These results were repeated with cells from multiple donors and also by studying phagocytosis with IgG-opsonized 1-μm latex beads (Fig. 1H).

Confirmation of phagocytosis by γδ T cells by TEM

To confirm these cells as γδ T cells, we performed TEM. Consistent with confocal findings, the cells were <10 μm (even smaller
from TEM preparation associated shrinkage) with typical lymphocyte morphology. Also seen were punctate collections of highly electron dense bodies up to 25 nm in diameter both on the cell surface and in endocytic compartments (Fig. 2A) representing the magnetic microbeads used to select γδ T cells which together with morphology distinguish γδ T cells from other cell types. The γδ T cells observed in this study and the pattern of γδ TCR staining, including clustering in endosomes, are consistent with previous electron micrograph studies of these cells (23). Few contaminating monocytes and only one neutrophil were seen with both displaying markedly different morphology (Fig. 2, B and C, respectively) and lack of electron dense anti-TCR-γδ microbeads. Bacteria were identified by their size (0.5–1 μm) and distinct morphology (Fig. 2D). Again, a small proportion of γδ T cells had formed pseudopod-like protrusions around adsorbed bacteria (Fig. 3A). Bacteria could be seen inside cells with distinct lymphocyte morphology and positive anti-TCR-γδ microbead staining at a frequency of 5–10% between sections (Fig. 3, B and C). Although these γδ T cells with phagocyted E. coli had a somewhat increased cytoplasmic volume reminiscent of monocytes, this was most likely due to increased swelling after ingestion. Moreover, these cells retained lymphocyte nuclear morphology and had microbeads present at the cell surface as well as intracellularly, whereas none of the monocytes or the neutrophil carried any microbeads despite extensive uptake of bacteria (Fig. 2, A–C). We speculate that the aforementioned dendritic population of γδ T cells are responsible for phagocytosis.

**Phagocytosis by γδ T cells is functionally linked to, and can be assayed by, Ag presentation on MHC class II**

Although it is intriguing that human γδ T cells can phagocytose, do they, like neutrophils, simply ingest and remove offensive material? In light of their ability to up-regulate MHC class II and present Ags to αβ T cells (5), we hypothesized that phagocytosis and Ag presentation are functionally linked in γδ T cells. To test this hypothesis and as an additional assay of phagocytosis, we conjugated whole influenza A virus matrix (M1) protein onto 1 μm polystyrene beads (M1 beads) and incubated these with freshly isolated nonactivated γδ T cells. Surface MHC class II loading of M1 peptide was detected by A1C5 murine T cell hybridomas which secrete murine IL-2 (mIL-2) in recognition of processed M1 peptide (18). When pulsed with anti-M1 rabbit antiserum opsonized M1 beads, the human peripheral blood γδ T cells could clearly process and present M1-derived peptides on surface MHC class II as detected by the mIL-2 ELISA (Fig. 4A). An equal number of unpulsed γδ T cells, as well as similarly purified freshly isolated human CD16+/CD56+ NK cells, were used as negative controls. Pulsed NK cells did not result in a significantly different presentation of M1 peptide to A1C5 hybridomas compared with unpulsed NK cells. To demonstrate active phagocytic uptake, γδ T cells were incubated with cytochalasin D 1 h before Ag pulsing. To
show that the Ag presentation was dependent on active Ag processing in the γδ T cells, chloroquine was added to inhibit acidification of the Ag processing and loading compartments. γδ T cells were also pulsed in the equivalent dilution of DMSO for control for any effect that the drug solvent might have on the assay. The addition of chloroquinol D and chloroquine reduced presentation of M1 peptide specific A1C5 hybridomas which was inhibited by chloroquine (Clq) and cytochalasin D (CyD). DMSO drug solvent control had no effect on M1 activation by pulsed γδ T cells. B, γδ T cells incubated with supernatants from M1 bead preparations were unable to activate A1C5 hybridomas so any Ag presentation must have been derived from phagocytosis of beads. The mean ± SD of triplicate Ag presentations is plotted. Cii, A blocking mAb (L243) against HLA-DR is able to completely block cytoxis of beads. The mean ± SD of triplicate Ag presentations is plotted.

**FIGURE 4.** Phagocytosis and Ag presentation in γδ T cells are functionally linked, a functional assay of phagocytosis. A, Unpulsed CD16+ NK T cells (white) and γδ T cells (dark gray) were very poor at activating A1C5 hybridomas. CD16+ NK T cells pulsed with anti-M1 opsonized M1 beads did not display significantly better presentation (p > 0.05) compared with unpulsed. Pulsed γδ T cells however, induced vigorous activation of M1 peptide specific A1C5 hybridomas which was inhibited by chloroquine (Clq) and cytochalasin D (CyD). DMSO drug solvent control had no effect on A1C5 activation by pulsed γδ T cells. B, γδ T cells incubated with supernatants from M1 bead preparations were unable to activate A1C5 hybridomas so any Ag presentation must have been derived from phagocytosis of beads. The mean ± SD of triplicate Ag presentations is plotted. Cii, A blocking mAb (L243) against HLA-DR is able to completely block any detectable presentation to the HLA-DRB1*0101-restricted M1 Ag-specific A1C5 hybridoma, whereas an isotype control is not. Cii, HLA-DRB*0102 and DRB*0101 γδ T cells were pulsed with whole heat-inactivated influenza A/PR/8/34 virus and used to present to A1C5 hybridomas. M1 peptide was only presented efficiently by DRB1*0101 γδ T cells (gray shade) and not by DRB1*0102 γδ T cells (white). The mean ± SD of triplicate Ag presentations is plotted.

**FIGURE 5.** Ag presentation of phagocytosed M1 beads is directly attributable to γδ T cells and is enhanced by Ab opsonization and activation of γδ T cells. Ai and Aii, γδ T cells (dark gray bars) were pulsed with naked M1 beads or anti-M1 opsonized beads (“Ig+M1 beads”) with or without activation and presentation was measured by A1C5 T cell hybridoma mIL-2 production. As negative controls (white bars), the same preparations devoid of γδ T cells were used. To control for monococyte contamination, parallel negative controls were spiked with 5 × 10^4 PBMCs containing an excess number of monocytes (light gray bars). **Bi**, Activation of γδ T cells. Bi, γδ T cells were sorted on TCR-γδ by flow cytometry reducing CD14+ monocyte contamination to <0.2%. Bii, Sorted cells were pulsed with M1 beads, activated and cocultured with A1C5 hybridomas (gray bar). The same activation coculture with M1 beads plus A1C5 hybridomas but devoid of γδ T cells (white bar) was used as a negative control. In the virtual absence of monocyte contamination, γδ T cells still retained Ag presenting capacity. The mean ± SD of triplicate Ag presentations is plotted.

βγ T cells are unable to support presentation of this epitope to this hybridoma (Fig. 4 Ai and Aii), as are HLA-DRB1*0701 LCL cells (supplementary Fig. 2). In these experiments, whole heat-inactivated nonopsonized influenza A/PR/8/34 virus was used as a source of M1 Ag for the presentation.

Monocyte contamination in γδ T cell preparations does not account for Ag presentation of phagocytosed M1 beads

Previous studies of MHC class II Ag presentation by human γδ T cells have not directly addressed the role of contaminating cell types such as monocytes in cell preparations (5). Neither have human γ982 T cells been directly shown to be responsible for the Ag presentation. To account for contaminating PBMCs, especially monocytes, parallel experiments were performed using PBMCs containing a number of monocytes in excess to the potential contaminating population (5 × 10^4), but devoid of γδ T cells and these confirmed that γδ T cells were responsible for the majority of the presentation seen (Fig. 5 Ai and Aii). Furthermore, γδ T cells were sorted on TCR-γδ expression by flow cytometry and thereby reducing monocyte contamination to <0.2% (Fig. 5 Bi). These γδ T cell preparations, used at 2.5 × 10^4, still retained their capacity to present phagocytosed Ag (Fig. 5 Bi). Moreover, the fact that these cells were purified with anti-TCR-γδ, indicates that the Vγ9δ2 subtype, or a subtype thereof, was responsible for the phagocytosis. This of course does not preclude the possibility that also other subtypes are capable of similar uptake and Ag presentation.
Activation and CD16 engagement increases phagocytosis and Ag presentation by γδ T cells

Freshly isolated γδ T cells display high expression of the phagocytic receptor CD16/FcγRIII (16, 25). Upon activation, these cells lose expression of CD16 while up-regulating MHC class II, CD80, and CD86 (5, 16, 25) (Fig. 6A). The proliferatively phagocytic DCs also express CD16 and likewise upon activation DCs also lose CD16 and up-regulate MHC class II, CD80, and CD86 to become potent presenters of phagocytosed Ags (26). We therefore tested whether CD16 engagement through opsonizing anti-M1 Abs and/or activation of γδ T cells improved phagocytosis and subsequent Ag presentation. γδ T cells were pulsed with M1 beads in the presence or absence of anti-M1 opsonization and then were either IPP activated or not. To assess the role of CD16, γδ T cells were also pulsed with nonopsonized M1 beads.

γδ T cells pulsed with nonopsonized M1 beads showed poor MHC class II presentation of M1 peptide to A1C5 hybridomas. γδ T cells pulsed with opsonized M1 beads and γδ T cells pulsed with nonopsonized M1 beads but which were subsequently activated by IPP were both able to present Ag to A1C5 hybridomas with comparable potency. Ig opsonization and IPP activation potentiated Ag presentation markedly, to a level greater than the sum of the two alone (Fig. 5A).

To further characterize the role of CD16 in phagocytosis and Ag presentation in γδ T cells, we compared the ability of CD16 high freshly isolated γδ T cells and CD16 low overnight IPP-activated γδ T cells to phagocytose M1 beads and present M1 peptide to A1C5 hybridomas. CD16 high freshly isolated γδ T cells were pulsed with anti-M1 opsonized M1 beads and were subsequently either activated with IPP or not. Freshly isolated γδ T cells from the same preparation were also directly activated overnight resulting in a CD16 low population, which were subsequently activated by IPP preactivated either IPP activated or not. To assess the role of CD16, γδ T cells were also pulsed with nonopsonized M1 beads (γδ**). Freshly isolated γδ T cells from the same preparation were also directly activated overnight resulting in a CD16 low population, which were subsequently activated by IPP preactivated either IPP activated or not (γδ**). Surprisingly, the CD16 low IPP-activated population of γδ T cells were able to process and present M1 peptides to A1C5 hybridomas better than CD16 high M1 pulsed γδ T cells. C, Ig opsonization enhancement of Ag presentation was partially abrogated by blockade with mouse IgG1 aCD16. Non-specific mouse IgG1 at the same concentration had no effect. The mean ± SD of triplicate Ag presentations is plotted.

Discussion

The ability to phagocytose large foreign bodies and process and present associated Ags on MHC class II is a hallmark of innate myeloid lineage cells such as monocytes and DCs and has never before been described in mammalian lymphocytes. We have shown in this study that freshly isolated γδ T cells are able to phagocytose both E. coli and 1 μm synthetic beads via the CD16 receptor. Only a proportion of γδ T cells observed had phagocytosed and we suspect that this is due to differences in their activation state or perhaps different subpopulations of Vγ9δ2 T cells present. Furthermore, we have shown that phagocytosis leads to Ag processing and presentation on MHC class II of peptides clearly derived from the phagocytosed complexes. The use of both confocal microscopy and TEM combined with anti-TCR-γδ staining ensured the accuracy of the observations despite the presence of a low degree of monocyte contamination. Previous TEM studies of γδ T cells also reported very similar structural observations (29). The monocyte contamination was also of concern for the Ag presentation experiments. To ensure that the γδ T cells were responsible for the Ag presentation of phagocytosed bead-bound material, we used monocytes in exceeding amounts in control experiments with resulting lower Ag presentations (Fig. 5A). As an additional control experiment, we showed that CD16⁺/CD56⁻ NK cells are unable to present M1 Ag to the same T cell hybridoma (Fig. 4). A previous study by Kang et al. (30) indicated that CD16⁺/CD56⁻ NK cells may be able to phagocytose. However, because these cells were only defined as NK cells on the basis of CD16 positivity, we suggest that they may well have been γδ T cells. The TEM images obtained by Kang et al. (30) showed cells with very similar ultrastructure to that of γδ T cells reported in this study and previously.

It has been proposed that T cells were the first lymphocytes to acquire variable-diversity-joining-type receptors following their evolution from myeloid and lympho-myeloid tissue (31). Recent work showed that the earliest thymic progenitors for T cells still retain myeloid lineage potential, whereas B cell progenitors do not (32, 33). In addition, phylogenetic studies strongly favor a scenario in which the δ TCR evolved before both the αβ TCR and B cell immunoglobulins (14). Because of their unique display of both myeloid and lymphoid lineage features, including phagocytosis, we speculate that the predecessors of γδ T cells represent the proposed proto-T cell evolutionary intermediate from which lymphoid cells arose from myeloid precursors (14). If this is correct, then it
is perhaps not surprising that these cells are capable of phagocytosis and professional Ag presentation. It is interesting to note that B lymphocytes in teleost fish and Xenopus have also been shown to phagocytose microbes (22), although it was not shown whether this can lead to Ag presentation. We speculate that the ability to phagocytose was retained after lymphocyte evolution from ancestral myeloid lineages in B cells in some animal groups and in γδ T cells in mammals further complicating simple interpretations of how the adaptive immune system evolved (34). Further studies of lymphocyte phagocytosis in different representative species should shed further light on this.

The functional consequences of these findings are far-reaching. It is clear that Ab-assisted opsonization and uptake into DCs involves the low affinity receptor for IgG CD16 (Fc γRIII) (35). Furthermore, this can lead to efficient and functionally important enhancement of MHC class II presentation (36, 37). Judging by anti-CD16 blocking experiments (Fig. 6C), a major part of the receptor-mediated phagocytic uptake into γδ T cells appears to also be CD16 mediated. Other receptors are also likely to be involved, as we found CD16 low IPP-activated γδ T cells were capable of phagocytic uptake and Ag presentation of M1 beads (Fig. 6D). We speculate that such uptake, apart from leading to Ag presentation, represents an important part of how γδ T cells sense and react to microbes. The tissue resident distribution of some subsets of γδ T cells (epithelial, including mucosal, tissues) would make them ideal for this purpose. TLR and related “sensing of nonself” molecules in endosomes and in the cytoplasm are known to be present and responsive in γδ T cells (38, 39), and could therefore represent an important regulating step in the encounter with an endocytosed or phagocytosed pathogen. It was recently demonstrated that mycobacteria infection activates and expands T cells in mammals further complicating simple interpretations of how γδ T cells are capable of expressing MHC class II molecules, and of functioning as antigen-presenting cells.

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References
against tumor cells is enhanced by monoclonal antibody drugs–rituximab and trastuzumab. *Int. J. Cancer* 122: 2526–2534.


