Human γδ T Lymphocytes Are Licensed for Professional Antigen Presentation by Interaction with Opsonized Target Cells

Nourredine Himoudi, Daniel A. Morgenstern, Mengyong Yan, Bertrand Vernay, Luisa Saraiva, Yin Wu, Cyrille J. Cohen, Kenneth Gustafsson and John Anderson

*J Immunol* 2012; 188:1708-1716; Prepublished online 16 January 2012;
doi: 10.4049/jimmunol.1102654
http://www.jimmunol.org/content/188/4/1708

Supplementary Material
http://www.jimmunol.org/content/suppl/2012/01/17/jimmunol.1102654.DC1

References
This article cites 32 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/188/4/1708.full#ref-list-1

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

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

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Human γδ T Lymphocytes Are Licensed for Professional Antigen Presentation by Interaction with Opsonized Target Cells

Nourredine Himoudi,* Daniel A. Morgenstern,* Mengyong Yan,* Bertrand Vernay,† Luisa Saraiva,‡ Yin Wu,‡ Cyrille J. Cohen,§ Kenth Gustafsson,‡,1 and John Anderson*,1

Activated human blood γδ T cells have also been previously demonstrated to behave as professional APCs, although the processes that control APC function have not been characterized. In this study, we show that the acquisition of potent APC function by human blood γδ T cells is achieved after physical interaction with an Ab-coated target cell, a process that we refer to as licensing. In cancer models, licensing of γδ T cells by tumor-reactive mAbs promotes the uptake of tumor Ags and professional presentation to tumor-reactive αβ T cells. We propose that licensing by Ab is a mechanism whereby the adaptive properties of γδ T cells are induced by their innate functions in a spatially and temporally controlled manner. The Journal of Immunology, 2012, 188: 1708–1716.

Professional APCs have the hallmark properties of taking up soluble Ag, or of sampling Ags from target cells by phagocytosis, and then presenting processed Ags in the context of MHC class II, or cross-presenting in the context of MHC class I, to induce primary immune responses in naïve Ag-specific αβ T cells. Dendritic cells (DCs), the prototypic professional APC, take up Ags at sites of infection, injury, or cancer and undergo a maturation step involving upregulation of costimulation molecules prior to migrating to sentinel lymph nodes. In this setting, an interaction with CD40L on the surface of CD4-positive T cells results in CD40 signaling in the DC, which is required for acquisition of full Ag presentation function, a process referred to as licensing (1–3).

γδ T cells typically represent 1–5% of T lymphocytes in human blood. In contrast to conventional αβ T cells, γδ T cells express a limited repertoire of TCR V-region genes; the most common subtype in human blood is Vγ9V δ2, which recognizes a group of nonpeptide phosphoantigens, for example isoprenyl pyrophosphate (IPP), associated with infection or cancer (4, 5). Stimulated γδ T cells undergo an activation, which results in a plethora of poorly defined changes including proliferation, proinflammatory cytokine and chemokine secretion, and altered cell surface phenotype (6). Activated human peripheral blood γδ T cells also have properties of innate immunity, including cytotoxicity mediated by NK receptors (7–11), Ab-dependent cell-mediated cytotoxicity (ADCC) (12, 13), and phagocytosis (14). Notably, we and others have shown FcγRIII (CD16) expressed on at least some human γδ T cells to be a potentially important molecule mediating innate effector functions (15, 16). These changes are likely to be subtype specific along classical T cell divisions; that is, T naïve, T central memory, T effector memory, and T effector memory RA+ (16).

At least one subtype of human γδ T cells has been shown to be able to express MHC class II, costimulatory molecules, and lymph node-homing chemokine receptors (e.g., CCL7) and has been characterized as a professional APC (17, 18). Previous studies have thus demonstrated the ability of γδ T APC to present and cross-present Ags from soluble protein (17–19), and we and others have shown that human blood γδ T cells can take up large fragments from intact cells by the processes of phagocytosis (14) or trogocytosis (20). Also, we found that Ags associated with these large (>1 μm) structures can be processed and presented, bound to MHC class II, by γδ T cells to other T cells (14). We therefore hypothesized that when activated γδ T cells are cocultured with transformed cells, the tumor cells would serve not only as targets for γδ T-mediated killing but also as a source of tumor-associated Ags (TAAs) for processing and professional presentation by the γδ T cells to tumor-reactive αβ T cells. In this study, we show that human γδ T cells do indeed present TAAs from tumor cells. Moreover, we have found that the process of APC function is tightly regulated, requiring interaction with cell surface-bound Ab, a requirement we refer to as licensing of γδ T cells for professional APC function.

Materials and Methods

Cell isolation

Fresh peripheral blood was centrifuged over Ficoll-Hypaque to obtain PBMCs according to the local ethical guidelines on experimentation with human samples. Vγ9V δ2 T cells were isolated by two rounds of purification using anti-γδ TCR (MicroBead Kit, which consists of a hapten-conjugated anti-TCRγδ Ab and FITC-conjugated anti-hapten microbe-
ads). γδ T APCs were generated from purified γδ T cells via stimulation for 24 h with 50 μM IPP (Sigma) in the presence of 200 U/ml IL-2.

**Cell lines and humanized Abs**

Daoudi and Raji (B lymphoma) and K562 (erythroleukemia) cell lines were maintained in RPMI 1640. Neuroblastoma cell line LAN-1 cells were maintained in culture with DMEM (BioWhittaker, Walkersville, MD), SK-Mel-23 cells were grown as monolayer cultures in F10–Ham medium, and SK-Mel-28 melanoma cell lines were grown in RPMI 1640 medium. All media were supplemented with 10% FBS and 2 mmol/l glutamine, in the absence of antibiotics, at 37°C and 5% CO2 humidified atmosphere. All cell lines were mycoplasma-free. Humanized mAbs, anti-CD2, IgG1 (Hu4.18K322A), anti-CD45RB, IgG1 (Thermo Scientific, Loughborough, U.K.), and anti-CD20 IgG1 (rituximab; Roche Pharma) were used at 10 μg/ml to opsonize the cell lines.

**Ag cross-presentation assays**

Pured γδ T cells were IPP activated for 24 h and cocultured for 5 d in the presence of 200 IU/ml IL-2, with opsonized tumor cells and CFSE-labeled β2 T cells at a ratio of 10:10:1 (γδ T cells/β2 T cells/tumor cells) in the presence of 70-Gy–irradiated HLA-A2– EBV–transformed B cells at a γδ T cell/feeder cell ratio of 5:1, or in presence of conditioned media prepared from β2 lymphoblastoid cell lines. The APCs were washed extensively before culture with responder cells. When required, tumor Ag was added in the coculture either as Daudi lystate (50 μg/ml), SK-Mel 23 lystate (50 μg/ml), recombinant PAX5 protein at 20 μg/ml (Caltag Medsystem, Milton Keynes, U.K.), or peptides PAX5 (TLPGYPHPV), PRAME (VLDGLDVLL), or Mart-1 (ELAGIGILTV) (ProImmune, Oxford, U.K.). All peptides were used at 5 μg/ml, and IL-2 (200 IU/ml) was added at day 3. In Transwell experiments, cells were separated by a 1-μm pore-size membrane. The upper chamber was carefully taken from the well, cells were harvested, washed, and FACS analyzed for CFSE dilution. For the intracellular cytokine stimulation, surface staining was performed by incubating cells with PE-conjugated pentamer raised against HLA-A*0201–restricted epitope TLPGYPHV derived from PAX5 (Proimmune). Cells were then permeabilized in 100 μl Cytofix/Cysteine solution (BD Biosciences) at 4°C for 20 min, washed with Perm/Wash buffer (BD Biosciences), and followed by intracellular staining with allophycocyanin-conjugated anti-human IFN-γ Ab (BD Biosciences) at 4°C for 30 min. Intracellular secretion of IFN-γ was assessed, with gating on the PAX5 γδ T clone pentamer-positive cells.

In some experiments (see Fig. 3E), γδ T cells were IPP activated for 24 h, cocultured with opsonized LAN-1 tumor cells, bead sorted, then rested or not in 50 IU/ml IL-2 for 24 h before reincubation with opsonized LAN-1 and responder β2 T cells in the presence of Daudi lystate as described earlier.

In blocking experiments, γδ T cells were blocked with a mouse monoclonal IgG1 anti-CD16 blocking Ab (clone LNK16; Abcam) or HLA-A2 neutralizing mAb (clone B7.7.2, purified from hybridoma; American Type Culture Collection) or monoclonal mouse IgG1 at 10 μg/ml for 30 min at 37°C/5% CO2 before pulsing with Daudi lystate. In the experiment comparing DCs and γδ T APCs, monocyte-derived DCs were derived from monocytes purified with CD14 microbeads (Miltenyi) and cultured for 6–7 d with 50 ng/ml GM-CSF and 10 ng/ml IL-4 (Peprotech). Mature DCs were pulse with either Mart-1 peptide, irrelevant PRAME peptide, or SK-Mel-23 tumor cell lysate for 2 h, then washed and added to the coculture with CFSE-labeled β2 T cells. In any individual experiment, the numbers of APCs, tumor targets, and T cells were kept constant.

**αβ CTL clones and αβ T cells transduction with a MART-1 TCR-expressing retrovirus**

High-avidity CTL clones raised against HLA-A2–restricted epitopes, TLPGYPHPV derived from PAX5 and VLDGLDVLL derived from PRAME, have been previously described (21, 22). To generate Ag-specific but Ag-inexperienced T cells, we transduced healthy donor PBMCs with a retrovirus expressing MART-1 TCR (containing a Vα12 chain) specific to an HLA-A*0201–restricted epitope, M26-35 (ELAGIGILTV). Briefly, TCR α- and β-chains were isolated from a highly lytic HLA-A2–restricted CTL clone recognizing the melanoma-associated Melan-A/MART-1 Ag and inserted into a retrovirus vector capable of robust and coordinated expression of the transgene. The retroviral vector and packaging construct pCL-Ampho (Imagenex) were cotransfected into 293 T cells using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer’s instructions. Culture supernatants containing retroviruses were harvested twice at 48 and 72 h after transfection and were concentrated prior to transduction. PBMCs from HLA-A*0201 healthy donors were stimulated in vitro at 1 × 10⁶ cells/well with Dynabeads coated with anti-CD3, anti-CD28, and anti-CD137 stimulating Abs in the presence of 300 IU/ml IL-2 for 3 d. T cell cultures were then transduced as follows: 24-well plates were coated with RetroNectin (Takara, Otsu, Japan), then preloaded with retroviruses according to the manufacturer’s instructions. T cells (2.6 × 10⁶) were added to each well in 1.3 ml Mart-1 TCR retroviral supernantant, supplemented with 300 IU/ml recombinant human IL-2, and the plates were centrifuged for 90 min at 1000 × g. The next day, the medium was replaced with fresh retroviral supernantant, and the centrifugation was repeated. Transduction efficiency and TCR expression on T cells were confirmed by flow cytometry using 10 μg/ml anti-TCR Vβ12 (Immunotech). The cells were rested for 24 h, after which transduced cells were CFSE labeled and assayed for Ag reactivity by 5-d coculture with DCs or with an activated γδ T cells in the presence of opsonized LAN-1 tumor cells and Ag (SK-Mel-23 tumor lystate, Mart-1 peptide [ELAGIGILTV], or irrelevant peptide). IL-2 was added at day 3.

**Confluent microscopy**

Opsonized Daudi cells were labeled either with Vybrant DiI (Invitrogen, U.K.) or CFSE and infected with IPP-activated γδ T cells stained with mouse anti-human TCR-γδ (BD Biosciences, Heidelberg, Germany) and secondary Ab goat anti-mouse Cy5 (Caltag, U.K.), or Alexa Fluor 488 phalloidin (Invitrogen, Grand Island, NY) before being immediately analyzed. Images were captured using LSM 510 confocal microscopy (Zeiss) with Leica confocal software.

**Cytotoxicity assays**

Freshly isolated or activated γδ T cells were used as effector targets. Target cells were coated or not with the opsonizing mAb and labeled with 100 μCi Na⁵¹CrO₄ in cell culture and tested in a standard chromium release assay as previously described.

**Activation phenotype of γδ T after coculture**

γδ T cells were activated by coculture with lymphoblastoid cell line conditioned media in the presence of 100 IU/ml recombinant human IL-2 and 50 μM IPP and cultured with opsonized or non-opsonized Daudi or Raji cell line for 72 h. γδ T cell phenotype was analyzed by FACS using the following mAbs: monoclonal mouse IgG2 PE-conjugated anti–HLA-DR, clone L243 (BD Biosciences); monoclonal mouse IgG1 PE-conjugated anti-CD86, clone FUN-1; anti-CD11c–allophycocyanin, clone B-ly6; anti-CD40–PE–Cy5, clone 5C3; and CD80–FITC, clone L307.4 (BD Biosciences). To characterize the γδ TCR phenotype, we used anti-Vγ9–PE–Cy5 (Beckman-Coulter) to stain activated Vγ9Vδ2 T cells and a pan anti-γδ T–biotin clone B1 (BD Pharmingen).

**Results**

**Cross-presentation of TAA by human blood γδ T cells**

As a model to study the capacity of human γδ T cells to interact with human cancer cells, we used immunomagnetic positive selection from peripheral blood, using a pan-γδ TCR Ab. Using this method, yields of γδ T cells were more than 98% pure (Fig. 1A), and percentage contamination with CD11c-positive cells was between 0.3 and 0.6% (Fig. 1B). As a tumor model, we initially used the Burkitt lymphoma cell line Daudi because we (Fig. 1C) and others (12) showed that opsonization with anti-CD20 IgG1 mAb (rituximab) enhances ADC of Daudi cells by γδ T cells, and because we showed that activated human blood γδ T cells could take up fragments from opsonized Daudi cells (Fig. 1D). In addition, we reasoned that opsonized Daudi cells could, in this model, serve as a source of TAA for processing and presentation on γδ T MHC to other T cells in a rituximab-dependent manner. To test this, we investigated cross-presentation of PAX5 Ag, a nuclear transcription factor expressed in B lineage cancer cells including Daudi (22, 23), by γδ T MHC class I. We have previously described a CD8 T CTL clone specific for the PAX5 peptide sequence TLPGYPHPV and restricted to HLA-A*0201 (22). We tested the ability of γδ T cells to present PAX5 Ag from intact tumor cells in a 5-d coculture involving 1) IPP-activated γδ T cells from an HLA-A*0201–positive donor, 2) rituximab-opsonized HLA-A*0201–negative Daudi cells, and 3) CFSE-labeled αβ CTL clone restricted to PAX5/HLA-A*0201 in the presence of
conditioned medium from a B lymphoblastoid cell line (Fig. 2A; see Supplemental Fig. 1 for lymphocytoid cell line detail). Robust proliferation of the αβ clone was individually dependent on γδ T cells and opsonized Daudi cells and was Ag- and MHC-restricted as demonstrated through use of an αβ CTL clone specific for HLA-A0201/PRAME (not expressed on Daudi cells) (Fig. 2B) and further demonstrated by lack of proliferation of the PAX5 clone when preincubating the γδ T cells with an HLA-A0201 blocking Ab (Fig. 2C). To exclude the possibility that contaminating professional APCs in the γδ T cell preparations were responsible for the observed stimulation, we titrated cells from the negative fraction after γδ TCR selection back into a coculture involving non-HLA-matched γδ T cells. No significant proliferation was observed until the number of negative fraction cells reached 20% (Supplemental Fig. 2). To confirm the interaction between IPP-activated γδ T cells and rituximab-opsonized Daudi cells, we used live imaging in which the γδ TCR was labeled with a fluorescent Ab and was found to form an aggregate at the immune synapses with Daudi cells. Moreover, a significant number of γδ T cells (8–13%) were found to be interacting with Daudi cells at any time point (Fig. 2D).

Ab-dependent licensing of γδ T APC by direct cell–cell contact

We wanted to compare the cross-presentation function of human γδ T cells after culture with either Ag-expressing intact tumor cells or with soluble tumor Ag. We also were interested in whether activation of γδ T cells for cross-presentation could cause bystander activation through release of soluble factors. To this end, we used Transwells with a 1-μm pore size restricting free diffusion to soluble proteins or cell fragments. In the presence of opsonized Daudi cells, γδ T cells could, as expected, cross-present PAX5 Ag to αβ CTL in an upper chamber, but the αβ CTL clone in the communicating lower chamber was only modestly stimulated (Fig. 3A). Similarly, a low level of activation of the CTL clone was seen if Daudi cells were not opsonized (Fig. 3B, upper panel) compared with the negligible background activation seen in the absence of Ag (Fig. 3B, lower panel).

Previous studies have demonstrated convincing Ag presentation to both CD4 and CD8 naïve αβ T cells by activated human γδ T cells loaded with soluble whole protein Ags (17–19). We hypothesized that the relatively weak APC function we observed in the absence of opsonized target cell was being greatly enhanced as a result of the interaction with Ab-coated cells. To test this hypothesis, we cultured activated γδ T cells and the PAX5 αβ CTL clone with a lysate from Daudi cells in the presence or absence of a third-party opsonized target cell (Lan-1 neuroblastoma cells, PAX5 nonexpressing, coated with anti-GD2 IgG1 mAb).
Whereas the presence of opsonized target Ag-negative cells induced a robust proliferation, we consistently observed only weak CTL response in the absence of opsonized cells (Fig. 3C, left panel). A control condition, in which Ab was omitted, resulted in loss of the profound APC function to the level seen in the lower chamber with Ag alone (Fig. 3C, middle panel). We refer to this dependency on direct contact with an Ab-coated target cell for induction of full APC function of γδ T cells as “licensing.” Consistent with previous reports (17), proliferation and IFN-γ secretion in the presence of Ag but absence of Ab was consistently greater than the background proliferation (Fig. 3C, right panel). γδ T cells after 5-d coculture stained homogeneously positively with a Vδ9 specific Ab. Results in panels B and C are representative of three independent experiments, and panels D and E are representative data of two separate experiments. Percentages shown in the FACS plots are the means of the percentages of viable positive cells from three experiments yielding similar results.

**Raji cells fail to license γδ T cells**

Because Daudi cells are well known to be efficiently killed by γδ T cells, we sought to investigate whether another very similar cell line, but one that is resistant to γδ T cell killing, can also license γδ T cells for more potent APC function. To do this, we used Raji cells, which others have described as being more resistant to γδ T cell cytotoxicity (24). We first confirmed that activated HLA-A*0201-positive γδ T cells are poor killers of anti-CD20-opsonized Raji targets. Notably, in contrast to Daudi cells, Raji cells failed to induce any APC function of γδ T cells in a 5-d coculture setting (Fig. 4A). We then sought to determine whether the failure of the induction of APC function of γδ T cells was the consequence of inadequate PAX5 Ag release from Raji cells. To do this, we repeated the 5-d coculture experiment using opsonized Raji cells in the presence of Daudi lysate as a source
FIGURE 3. Human blood γδ T cells are licensed for Ag cross-presentation by interaction with opsonized cells. A, αβ T cell clone activation determined by CFSE dilution and IFN-γ release in top and bottom chambers of a 1-μm-pore Transwell experiment. Daudi targets were opsonized and repeatedly washed before being added to the top chamber. B, Controls for the three-cell coculture protocol depicted in Fig. 2 in which Daudi cells alone or rituximab alone were used as the licensing agent. C and D, Five-day coculture 1-μm-pore Transwell experiment set up as depicted in the scheme. E, Human γδ T cells were activated with IPP in the absence of PAX5 Ag and then licensed with Lan-1 cells opsonized with anti-GD2 mAb for 2 d before purifying with two rounds of positive selection. Licensed γδ T cells were then rested for 24 h in the presence of IL-2. Twenty-four-hour rested γδ T cells were cocultured with labeled αβ CTL responder clone in the absence (central panel) or presence (right panel) of anti-GD2 opsonized Lan-1 cells. Positive control (left panel) were non-sorted licensed γδ T cells cocultured with responders and Ag. Typical FACS plots are shown from three different experiments performed in triplicate with two different donor cells each, and percentages shown in the FACS plots are the means of the percentages of viable positive cells from three experiments yielding similar results.
of PAX5 Ag. Despite the presence of high amount of soluble PAX5 Ag, opsonized Raji cells failed to license completely γδ T cells for APC function (Fig. 4B). Surprisingly, both opsonized and non-opsonized Daudi and Raji cells induce a strong upregulation of MHC class II (DR), CD40, and costimulatory molecules (Fig. 4C) suggesting that the licensing of γδ T cells for Ag presentation and the upregulation of molecules required for APC function are distinct events. Thus, Raji cells do not only resist γδ T cell killing but also fail to license them, independently of MHC class II and costimulatory molecule induction on the γδ T cells.

Licensed γδ T cells can present TAAs to TCR-transduced naive primary T cells

A hallmark of professional APCs is the ability to stimulate naive CTL. To generate a population of Ag-inexperienced human T cells of a single antigenic specificity, we transduced T cells from an HLA-A*0201–positive donor with a retrovirus encoding a TCR with specificity for an immunodominant HLA-A*0201–restricted peptide from the melanoma Ag MART-1 (Fig. 5A). CFSE-labeled MART-1 TCR-transduced T cells were cocultured with either γδ T cells (licensed by the presence of anti-GD2–opsonized Lan-1 cells) or with conventional DCs, both derived from the same HLA-A*0201–positive donor. The Ag was either MEL-23 melanoma cell line lysate or soluble MART-1 peptide. Licensed γδ T cells were found to present either Ag to the same degree as monocyte-derived DCs (Fig. 5B). Opsonization of target cells was mandatory to license γδ T cells for potent APC function (Fig. 5C).

We speculated that interaction of γδ cells with Fc receptors might be critical for the licensing phenomenon and therefore stained activated γδ T cells with Abs against the FcγRIII (CD16), CD32, and CD64, of which only CD16 was positive (data not shown). We then found that preincubating γδ T with a blocking Ab directed against the FcγRIII (CD16) abrogated the licensing
Therefore, the acquisition of full APC function in human peripheral blood γδ T cells requires a licensing event that involves a physical interaction with an Ab-coated target cell, and our data are consistent with a CD16–Fc interaction being essential for licensing.

**Discussion**

We have confirmed the finding of previous studies that, given appropriate stimulation, phosphoantigen-activated human blood γδ T cells, which overwhelmingly express the Vγ9Vδ2 TCR, can take up and cross-present exogenous Ag to Ag-experienced T cells.
or inexperienced αβ T cells. For the first time to our knowledge, we show that this process is subject to regulation, and we have identified one mechanism of likely important physiological significance in the host response to cancer. We have reproducibly seen, using two different antigenic systems and three different “licensing” opossumized cells, that the degree of Ag-specific stimulation of responder αβ T cells is massively increased in the presence of opossumized cells. In the presence of Ab-coated cells, the degree of stimulation is equivalent to that seen with mature Ag-loaded DCs, whereas in the absence of licensing, a more modest degree of stimulation above background is apparent.

Physiologically, we propose that the requirement for Ab coating of target cells provides an important regulatory circuit between innate and adaptive immunity, which will localize functional antigenic sampling of γδ T cells to their site of activation (Fig. 5D). Hence in the cancer models demonstrated here, activation of circulating γδ T cells at the tumor site by TCR ligand will activate their expansion and innate killing. However, full APC function will only be engaged after the binding of Ig to tumor cells. This in turn will both activate γδ T-mediated killing (by ADCC) (Fig. 1C) and license the cells for Ag presentation. It is not yet clear, however, whether γδ T cells kill their target in the process of being licensed. In this regard, it is of interest that there is a correlation between sensitivity to cytotoxic killing (in Daudi and Raji cells, respectively) and licensing ability. This differential sensitivity to killing has been reported previously, but the precise mechanism is not known although is speculated to be related to expression of an unknown ligand for Vγ9Vδ2 TCR (25). The differential expression of F1-ATPase related protein (putative ligand for Vγ9Vδ2 TCR) on Daudi and Raji cells might also contribute to the differential sensitivity (26). In support of the physiological significance of this concept in cancer are data documenting infiltration of γδ T cells into a broad array of tumor types (27–29), which when analyzed have been found to be predominantly of the Vγ9Vδ2 subtype (7, 9, 30, 31).

 Licensing of DCs by CD40 ligation is a distinct process from their maturation, the latter involving upregulation of costimulatory molecules, MHC class II, and chemokine receptors. Analogously, for IPP-activated γδ T cells, we found that upregulation of costimulatory molecules and HLA-DR was dependent on interaction with tumor target cells but independent of interaction with Ab. Moreover, activation via ligation of the TCR was not sufficient for full upregulation of costimulatory molecules or HLA-DR (Fig. 4C). The signaling that results in upregulation of costimulatory molecules on γδ T cells is not known, nor is it known whether it requires direct interaction with and/or killing of a target cell.

Cells with combined innate killing and Ag presentation function are well known; for example, macrophages. We have previously demonstrated the capacity of γδ T cells to take up and present Ags, using phagocytosis (14), and γδ T cells also take up Ag by trogocytosis (20). Hence, at the tumor site, γδ T cells have the potential to kill by innate cytotoxic mechanisms releasing Ag into solution and to take up Ags directly by phagocytosis, trogocytosis, or pinocytosis. Ab coating of target cells potentially enhances all these processes but additionally provides a distinct licensing signal for potent activation of tumor-reactive CTL.

It is interesting to speculate how licensing could regulate APC function spatially and temporally within the setting of response to cancer or infection. Previous data have demonstrated that Vγ9Vδ2 TCR activation induces upregulation of secondary lymphoid tissue chemokines CCR7 and CCR4 and enhanced lymphoid tissue-homing capabilities, as well as clustering within germinal centers of B cell follicles (32). However, regardless of the role of γδ T licensing in physiological systems, the availability of clinical reagents for γδ T activation and therapeutic mAbs to effect licensing in the tumor niche suggests new approaches toward Ag-specific vaccination/treatment for cancer and infection.

Disclosures
The authors have no financial conflicts of interest.

References


B lymphoblastoid cell lines provide essential factors for effective cross presentation by γδ T cells. A co-culture experiment involving a 1μm pore transwell. Proliferation of a CFSE labeled αβ CTL clone with specificity for PAX5 antigen is read out of cross presentation of antigen derived from opsonized Daudi cells. Conditioned media from B-LCL or the presence of B-LCL in the upper chamber provide essential factors for full cross presentation even in the presence of opsonized target cell. Experiments were repeated three times with similar results.
Contaminating DC in γδ-T cell preparations do not contribute significantly to the observed cross presentation. 5 day co-culture experiment in which CFSE labelled CTL clone specific for PAX5 is cultured with γδ-T cells and Rituximab-coated Daudi cells. Cells in the negative fraction following γδ-T cell bead selection from an HLA-A*0201 positive donor were added to HLA-A*0201 negative or positive γδ-T cells. Percentage CD11c positive cells in γδ-T cell preparations was always less than 1%. Data are representative of two different experiments with two different buffy coats each.
γδ-T cells from an HLA-A*0201 positive donor were cultured for 5 days with CFSE labelled PAX5 + HLA-A*0201 restricted CTL clone in the presence of soluble recombinant PAX5 antigen or BSA, and in the presence or absence of PAX5 negative licensing cell (anti-GD2-opsonized Lan-1). Representative FACS plots from two experiments performed in triplicates are shown.

Suppl Fig 3