The Journal of Immunology

RESEARCH ARTICLE | FEBRUARY 15 2004

Liver Dendritic Cells Present Bacterial Antigens and Produce Cytokines upon *Salmonella* Encounter [1]

Cecilia Johansson; ... et. al

J Immunol (2004) 172 (4): 2496–2503.
https://doi.org/10.4049/jimmunol.172.4.2496

Liver Dendritic Cells Present Bacterial Antigens and Produce Cytokines upon *Salmonella* Encounter[1]

Cecilia Johansson* and Mary Jo Wick[2]*†

The capacity of murine liver dendritic cells (DC) to present bacterial Ags and produce cytokines after encounter with *Salmonella* was studied. Freshly isolated, nonparenchymal liver CD11c$^+$ cells had heterogeneous expression of MHC class II and CD11b and a low level of CD40 and CD86 expression. Characterization of liver DC subsets revealed that CD8α^-CD4$^-$ double negative cells constituted the majority of liver CD11c$^+$ (~85%) with few cells expressing CD8α or CD4. Flow cytometry analysis of freshly isolated CD11c$^+$ cells enriched from the liver and cocultured with *Salmonella* expressing green fluorescent protein (GFP) showed that CD11c$^+$ MHC class II^{high} cells had a greater capacity to internalize *Salmonella* relative to CD11c$^+$ MHC class II^{low} cells. Moreover, both CD8α^- and CD8α^+ liver DC internalized bacteria with similar efficiency after both in vitro and in vivo infection. CD11c$^+$ cells enriched from the liver could also process *Salmonella* for peptide presentation on MHC class I and class II to primary, Ag-specific T cells after internalization requiring actin cytoskeletal rearrangements. Flow cytometry analysis of liver CD11c$^+$ cells infected with *Salmonella* expressing GFP showed that both CD8α^- and CD8α^+ DC produced IL-12p40 and TNF-α. The majority of cytokine-positive cells did not contain bacteria (GFP$^-$) whereas only a minor fraction of cytokine-positive cells were GFP$^+$. Furthermore, only ~30–50% of liver DC containing bacteria (GFP$^+$) produced cytokines. Thus, liver DC can internalize and process *Salmonella* for peptide presentation to CD4$^+$ and CD8$^+$ T cells and elicit proinflammatory cytokine production upon *Salmonella* encounter, suggesting that DC in the liver may contribute to immunity against hepatotropic bacteria. *The Journal of Immunology,* 2004, 172: 2496–2503.

The dual role of the liver in inducing tolerance to orally acquired Ags and its ability to support effector T cells against hepatotropic pathogens underscores the complex role of this organ in the immune system. Nonparenchymal cells of the liver include a significant population of T lymphocytes, a minor fraction of B lymphocytes, and innate cell populations such as NK and NKT cells (1). The liver also contains cells with Ag presentation capacity such as sinusoidal endothelial cells, Kupffer cells (resident macrophages), and dendritic cells (DC)[3] (1–4). In some cases, hepatocytes can even function as APCs (5).

DC are APCs that play a pivotal role in initiating and directing an immune response (6). The capacities of DC depend on their state of maturation, in which immature DC have a high capacity to internalize and process Ags, and mature DC are efficient stimulators of naive T cells. This latter function is attributed to increased expression of MHC, adhesion, and costimulatory molecules on mature relative to immature DC (6).

DC traffic through the liver in the blood that passes through this organ from the intestinal tract and the circulation. They access hepatic lymphatic vessels and enter the celiac lymph nodes (7). This blood-lymph translocation in the sinusoids is MHC-independent for both immature and mature DC and may be mediated by binding to Kupffer cells (8). In addition to their presence in lymph nodes draining the liver, DC are located within the liver in the portal areas and perivenular regions (2, 4).

DC in mice, which are typically identified by the surface molecules CD11c and MHC class II, have been divided into subsets based on expression of molecules including CD4, CD8α, and CD11b (9). CD8α^+CD4$^-$CD11b$^-$, CD8α^-CD4$^+$CD11b$^+$, and CD8α^-CD4$^-$CD11b$^+$ DC populations are present in the mouse spleen whereas peripheral lymph nodes contain an additional CD8α^-CD11b$^-$ population (9, 10). CD11c$^+$ cells in murine Peyer's patch (PP) do not express CD4 and are composed of CD8α^+CD11b$^-$, CD8α^-CD11b$^+$, and an abundant population of CD8α^-CD11b$^-$ cells that is only a minor subset of splenic DC (10, 11). Cells expressing CD8α or CD4 are present among CD11c$^+$ nonparenchymal cells in the liver (4, 12, 13).

Distinct functions, including the capacity to produce cytokines, present Ags, and induce tolerance, have been attributed to the different DC subsets from a given lymphoid organ (9, 10, 14–23). Data suggest that factors in the environment and/or the stimulus itself may influence the function of DC subsets rather than the subsets per se having distinct intrinsic functions (21, 24–27). DC from different organs, despite being the same subset, have also been shown to have a differential capacity to produce cytokines. For instance, PP DC produce IL-10 rather than IL-12 and promote a Th2-biased T cell response whereas splenic DC produce IL-12 but not IL-10 and induce a Th1-biased response (10, 28). Likewise, DC derived from cultured progenitors in the liver stimulated allogeneic T cells to produce IL-10 and IL-4 rather than IFN-γ (29). The mechanisms underlying these organ-specific functional differences in DC are not yet understood.

Salmonella enterica serovar Typhimurium (*S. typhimurium*) is a facultative intracellular bacterial pathogen with the capacity to survive and replicate in macrophages. *Salmonella* can also induce

*Department of Cell and Molecular Biology, Section for Immunology, Lund University, Lund, Sweden, and †Department of Clinical Immunology, Göteborg University, Göteborg, Sweden

Received for publication June 30, 2003. Accepted for publication December 12, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from the Swedish Research Council (Project 621-2001-1720) and the Swedish Foundation for Strategic Research.

[2] Address correspondence and reprint requests to Dr. Mary Jo Wick, Department of Clinical Immunology, Göteborg University, Guldhedsgatan 10, 413 46 Göteborg, Sweden. E-mail address: mary-jo.wick@immuno.gu.se

[3] Abbreviations used in this paper: DC, dendritic cell; PP, Peyer's patch; MLN, mesenteric lymph node; GFP, green fluorescent protein; FL, Flt3 ligand; CCD, cytochalasin D; 7-AAD, 7-aminoactinomycin D.

0022-1767/04/$02.00

death in infected cells by apoptotic as well as nonapoptotic mechanisms (30), and has a cytotoxic effect on phagocytes during infection (31). Macrophages induced to undergo apoptotic death upon infection with *Salmonella* expressing the type III secretion system, encoded by *Salmonella* pathogenicity island-1, are reservoirs of bacterial Ags that can be presented by bystander DC (32). Pathogenicity island-1-encoded genes as well as the capacity to activate caspase 1 are required for virulence of orally acquired *Salmonella* (33, 34) although they appear dispensable for induction of $CD8^+$ T cells (35).

S. typhimurium resides in the liver, the spleen, and mesenteric lymph nodes (MLN) after oral infection (31, 36–38). Furthermore, splenic DC harbor *Salmonella* during infection and present bacterial Ags to T cells (18, 39, 40). Although the liver supports effector T cells against hepatotropic bacteria such as *Listeria* and *Salmonella* (Refs. 41–43 and A. C. Kirby and M. J. Wick, manuscript in preparation), little is known about the capacity of liver DC to process bacteria for Ag presentation. Moreover, the cytokine production profile of liver DC, and liver DC subsets, upon bacterial encounter is unknown. The present study examines these issues using freshly isolated liver DC and *Salmonella*. Elucidating these parameters of liver DC function upon *Salmonella* encounter provides insight into their role in the immune response to hepatotropic bacteria.

Materials and Methods

Mice

BALB/c, C57BL/6, and OT-I (44) mice were bred in the animal facilities at Lund University (Lund, Sweden). DO11.10 mice (45) were bred and housed at Active Biotech, Lund, Sweden.

Bacterial strains and infection of mice

S. typhimurium 14028r harboring pJLP-2H-Kan (46), which encodes the Crl-OVA fusion protein, pJLP-1E-Kan (46), which encodes the Crl-HEL fusion, or pOVA (47) encoding OVA were used. *S. typhimurium* χ4550 Δ*asdA1* harboring pYA3259rOVA or pYA3259rOVA-green fluorescent protein (GFP) (39) were also used as specified. 14028r containing pJLP-2H-Kan or pJLP-1E-Kan were grown in Luria-Bertani broth or on Luria-Bertani agar plates supplemented with 50 μg/ml kanamycin overnight at 37°C. When pOVA was used, carbenicillin was substituted for kanamycin. χ4550 containing pYA3259rOVA (called χ4550/OVA) or pYA3259rOVA-GFP (called χ4550/OVA-GFP) were grown in Luria-Bertani broth without antibiotics (48).

Bacterial suspensions were prepared by removing colonies from agar plates into PBS, pH 7.4 (Life Technologies, Paisley, U.K.). Liquid cultures were centrifuged 1700 $\times$ *g* and the pellet was resuspended in PBS. The bacterial concentration was quantitated spectrophotometrically by determining the OD_{600}. The suspension was centrifuged, resuspended, and diluted in IMDM (Life Technologies) without antibiotics to be used in Ag processing assays, bacterial uptake assays, or to detect intracellular cytokines.

Flow cytometry

Flow cytometry analysis was performed using a Becton Dickinson FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). Abs from hybridomas 2.4.G2 (anti-FcRγII/III), M5/114 (anti-MHC class II), GK1.5 (anti-CD4), and YTS.169 (anti-CD8α) (Ref. in 39) were used. Abs were purified from supernatants using GammaBind Plus columns (Amersham Biosciences, Uppsala, Sweden) and were labeled with biotin or FITC (both from Sigma-Aldrich, St. Louis, MO). FITC-labeled anti-CD11c (HL3), PE-labeled anti-CD4, CD8α, CD11c, Gr-1, NK1.1, MHC class II, anti-IL-12p40 (C15.6) and anti-IL-10 (JES5-16E3), allophycocyanin-labeled anti-CD4 and CD8α and biotinylated anti-CD11b Abs were purchased from BD PharMingen (San Diego, CA). Biotinylated anti-TNF-α Ab was purchased from Caltag Laboratories (Burlingame, CA). Streptavidin-allophycocyanin (BD PharMingen) was used as the second step reagent. 7-Aminoactinomycin D (7-AAD; Sigma-Aldrich) was used in all samples to exclude dead cells. Incubations with Abs or reagents were for 20 min in the dark on ice in HBSS (Life Technologies) containing 3% FCS, 2 mM EDTA, and 0.01% sodium azide.

Isolation of liver DC

Liver DC were purified from naive mice or from mice injected i.p. for 9 consecutive days with 10 μg of recombinant human Flt3 ligand (FL; kindly provided by Amgen, Seattle, WA) as specified in individual experiments. Livers were perfused with 10 ml of PBS before removal from mice. The livers were then cut into pieces and digested with 0.8 mg/ml of collagenase type IV (Sigma-Aldrich) and 1 mg/ml DNase 1 (Sigma-Aldrich) in HBSS with constant stirring for 45–60 min at 37°C. The cells were washed twice, resuspended in PBS and mixed with 100% Percoll (Amersham Biosciences) to a 28% Percoll solution. This was overlaid on an 80% Percoll layer and centrifuged at 720 $\times$ *g* for 20 min. The lymphocyte layer was collected, washed, and the cells were labeled with anti-CD11c (N418) magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) and enriched in an AutoMACS (Miltenyi Biotec) or on Midimax columns (Miltenyi Biotec) following the manufacturer's protocol. The concentration of cells was estimated by trypan blue exclusion and purity was determined by the Ab HL3 (anti-CD11c) in flow cytometry analysis. $CD11c^+$ cells ≥90% pure (often ~98% pure) were used in experiments.

Intracellular cytokine staining and detection of GFP^+ cells

A total of 1–3 $\times$ 10^6 liver DC in Ultra-Low cluster 24-well tissue culture plates (CoStar-Corning, Cambridge, MA) were infected with *S. typhimurium* χ4550/OVA or χ4550/OVA-GFP at a bacteria to DC ratio of 10:1, 50:1, or 250:1. After centrifugation at 270 $\times$ *g* for 4 min, the plates were incubated for 2 h at 37°C. In some experiments DC were pretreated with 10 μg/ml cytochalasin D (CCD; Sigma-Aldrich) for 45 min at 37°C before the addition of bacteria and this was present for the duration of the 2 h coculture at 5 μg/ml. Following the bacterial coculture, the cells were washed four times in HBSS and were used in flow cytometry analysis to detect GFP^+ bacteria.

For uptake of GFP^+ bacteria in vivo, mice were infected with 10^8 bacteria i.v. and after 4 h organs were removed. This dose and time point were determined to be optimal for detecting GFP^+ cells without compromising the health of the animals (18). DC were isolated as previously described and GFP^+ $CD11c^+$ cells were analyzed by flow cytometry.

When cytokine production by *Salmonella*-infected liver DC was analyzed, the cells were incubated for 5 h with 5 μg/ml brefeldin A (Sigma-Aldrich) after the 2 h coincubation with bacteria and washing. The cells were stained for surface molecules and were fixed in 2% paraformaldehyde or formaldehyde for 20 min at room temperature. Fixed cells were washed and resuspended in permeabilization buffer (HBSS containing 0.5% BSA (Sigma-Aldrich), 0.5% saponin (Sigma-Aldrich), and 0.05% azide) for 20 min at room temperature. Staining for intracellular cytokines was performed for 30 min at room temperature in permeabilization buffer. The cells were then analyzed by four-color flow cytometry.

For detection of IL-10 by ELISA, an OptEIA mouse IL-10 set was used (BD Biosciences).

Ag processing and presentation assays

A total of 2 $\times$ 10^5 liver DC were seeded in 96-well plates in IMDM containing 10% FCS. Titrated numbers of 14028r/Crl-OVA, 14028r/Crl-HEL, or 14028r/OVA were then added to triplicate wells. As a positive control, the K^b-binding $OVA_{257–264}$ peptide or the I-A^d-binding $OVA_{323–339}$ peptide was added at the indicated concentrations. After 2 h, the cells were washed at least three times in HBSS and were left in IMDM containing 10% FCS and 50 μg/ml gentamicin. Finally, either 1–2 $\times$ 10^5 $K^b/OVA_{257–264}$-specific $CD8^+$ T cells from OT-I mice (44) or 1–2 $\times$ 10^5 I-$A^d/OVA_{323–339}$-specific $CD4^+$ T cells from DO11.10 mice (45) were added. Before addition, OT-I and DO11.10 T cells were MACS-purified from spleen or MLN using anti-CD8α or anti-CD4 biotinylated Abs, respectively, and streptavidin-conjugated beads. The T cells were 95–98% pure as determined by flow cytometry. After 64 h of incubation at 37°C, cultures were pulsed with [^{3}H]thymidine for 8 h and incorporation into cellular DNA was determined.

For experiments in which the DC were titrated, 0.8–2 $\times$ 10^6 liver DC were seeded in Ultra-Low cluster 24-well tissue culture plates (CoStar-Corning) in IMDM with 10% FCS and incubated for 2 h with *S. typhimurium* 14028r/Crl-OVA, Crl-HEL or OVA at the bacteria to DC ratios indicated in individual experiments. The cells were washed in HBSS and resuspended in IMDM containing 10% FCS and 50 μg/ml gentamicin. DC were then seeded in triplicate wells of 96-well plates and were diluted 2-fold into IMDM containing 10% FCS and 50 μg/ml gentamicin. Purified OT-I or DO11.10 T cells were added and proliferation was measured as previously indicated.

Results

Surface molecule expression on liver DC from naive and FL-injected mice

To characterize the surface phenotype of the liver DC used throughout these studies, flow cytometry analysis of freshly isolated, MACS-purified DC from the liver of either naive or FL-injected mice was performed (Fig. 1). Analysis of CD4 and CD8α expression on the CD11c$^+$ cells, MACS-enriched from the Percoll fraction of liver lymphocytes from naive mice, revealed that the vast majority expressed neither of these molecules (Fig. 1, *A* and *B*). That is, ~85% of CD11c$^+$ liver cells were CD8α^-CD4$^-$ whereas ~10% were CD8α^+ and ~5% were CD4$^+$. Analysis of CD11c$^+$ liver cells from FL-injected mice revealed that ~25% were CD8α^+ and very few were CD4$^+$ (Fig. 1, *A* and *B*). Analysis of CD11b expression on CD11c$^+$ liver cells from naive and FL-injected mice revealed a heterogeneous distribution of CD11b, with greater heterogeneity apparent on liver DC from naive mice (Fig. 1, *A* and *B*). The CD11b$^-$CD8α^- population that constitutes ~40% of CD11c$^+$ cells in the liver of naive mice is present as a smaller fraction of CD11c$^+$ in the spleen (~15%) (Fig. 1*A*). This population comprises a major part of CD11c$^+$ cells in PP and MLN (Ref. 10 and data not shown). The lack of a significant CD4$^+$ population and expression of CD11b and costimulatory molecules on the CD11c$^+$ cells isolated from the liver (Fig. 1) suggests that these are conventional rather than plasmacytoid DC (13). Lack of a significant B220$^+$ population in the purified cells is also consistent with this suggestion (data not shown).

Analysis of MHC and costimulatory molecule expression on freshly isolated liver DC revealed somewhat heterogeneous MHC class II expression on liver DC from naive and FL-injected mice (Fig. 1*C* and Fig. 2*A*). Liver DC from FL-injected mice had higher surface expression of CD86 and more heterogeneous CD40 expression compared with liver DC from naive mice (Fig. 1*C*).

CD8α^-, CD8α^+, and MHC class II^{high} liver DC can phagocytose Salmonella

Immature DC can phagocytose bacteria including *Salmonella* (18, 46, 49). To examine the phagocytic capacity of liver DC, freshly isolated liver DC were coincubated with GFP-expressing *S. typhimurium* for 2 h. Analysis of GFP in gated CD8α^- or CD8α^+ liver DC revealed that both CD11c$^+$CD8α^- and CD11c$^+$CD8α^+ could phagocytose *Salmonella* (Fig. 2 and Fig. 3). This was observed with DC purified from naive or FL-injected mice. Examination of GFP$^+$ cells within gated MHC class II^{high} or class II^{low} cells among liver DC from naive mice showed that CD11c$^+$ cells with high MHC class II expression could phagocytose *Salmonella* (Fig. 2*B*). In contrast, CD11c$^+$ MHC class II^{low} cells had relatively little ability to internalize bacteria. The dramatic reduction in the percentage of GFP$^+$ cells when the bacteria were cocultured with DC in the presence of CCD showed that the vast majority of the GFP was due to bacterial uptake rather than bacteria attaching to the cell surface (Figs. 2*B* and 3*B*).

Experiments were performed to address the capacity of liver DC to phagocytose *Salmonella* in vivo. In initial experiments, mice were infected orally with GFP-expressing *Salmonella*, and the presence of GFP$^+$ cells among MACS-purified DC from Percoll-enriched liver cells was analyzed by flow cytometry. In these experiments, GFP$^+$ events from orally infected mice were found at an extremely low frequency (<0.1% of purified liver DC), making reliable quantitation of GFP$^+$ liver DC after oral infection by flow

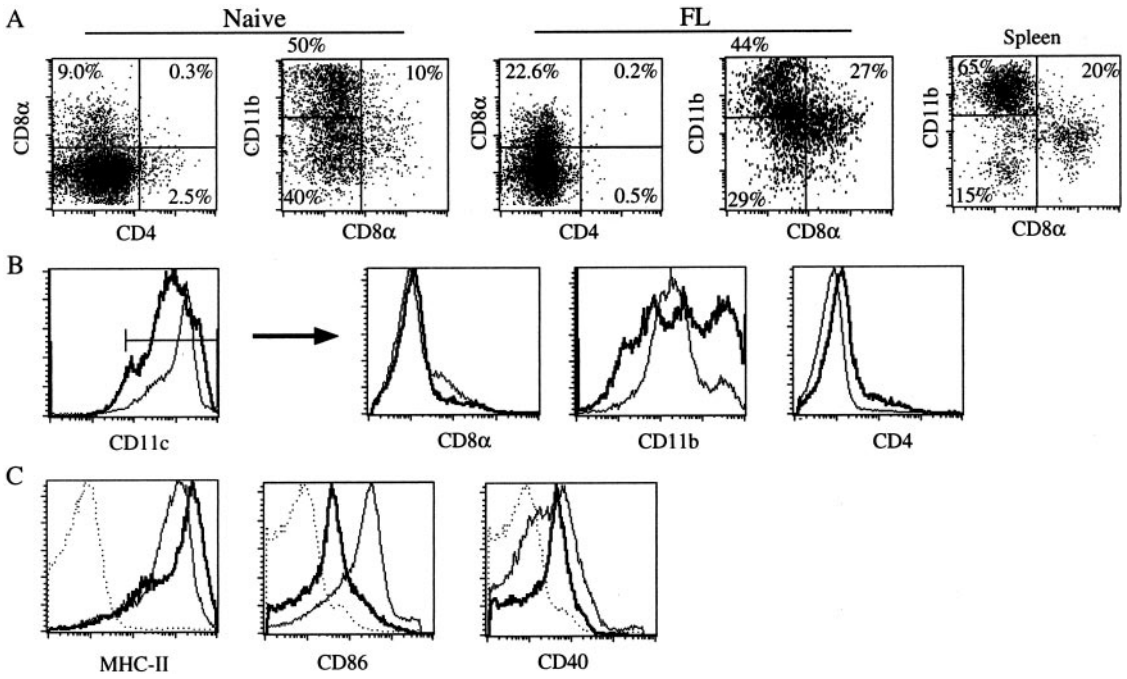

FIGURE 1. Surface marker expression on freshly isolated, MACS-purified liver DC from naive and FL-injected mice. Liver DC were purified as described in *Materials and Methods* and were stained with 7-AAD and for surface expression of CD11c, CD8α, and CD11b or CD4 and analyzed by four-color flow cytometry. *A*, Dot plots of viable (7-AAD$^-$) CD11c$^+$ liver DC from naive and FL-injected mice are shown. Both axes represent log fluorescence intensity. The data are from DC pooled from 10–20 naive or two FL-injected mice per experiment and are representative of seven independent experiments. The percentage of cells in each quadrant is indicated. *A*, CD8α and CD11b expression (*right* dot plot) on gated CD11c$^+$ cells isolated from the spleen (as described in Ref. 18) is shown for comparison. *B*, Histograms of the indicated surface molecules on liver DC from naive (thick line) or FL-injected (thin line) mice are shown. *C*, Histograms of the surface expression of MHC class II, CD86, and CD40 on the gated CD11c$^+$ cells in *B* are shown. Thin lines represent DC from FL-injected mice, thick lines represent DC from naive mice, and dotted lines are isotype-matched control Abs. One representative experiment of seven is shown.

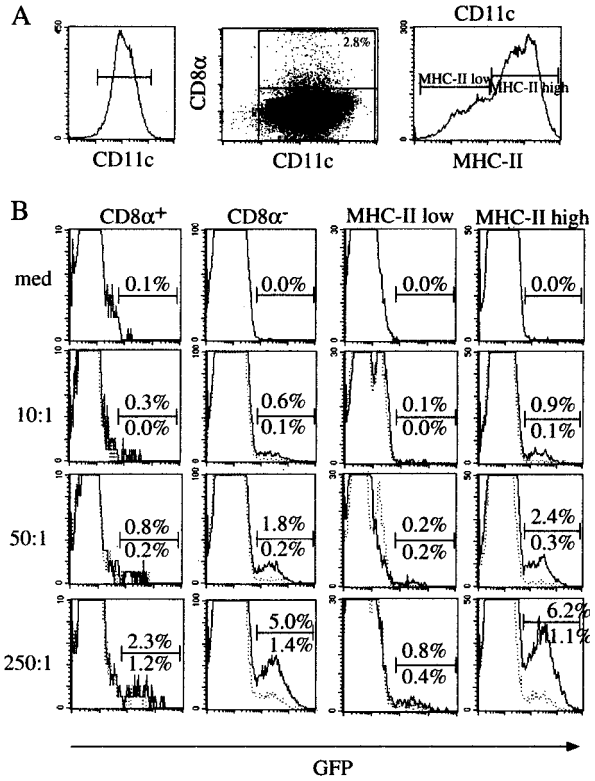

FIGURE 2. Uptake of *Salmonella* by liver DC from naive mice. *A*, Freshly isolated, MACS-purified, viable (7-AAD$^-$) liver DC from naive mice were stained for surface expression of CD11c and CD8α or MHC class II and analyzed by flow cytometry. The *right* histogram shows MHC class II expression on gated CD11c$^+$ cells. *B*, Liver DC were incubated in medium alone (med) or were cocultured with *S. typhimurium* χ4550/OVA-GFP for 2 h at the indicated bacteria to DC ratios. Following washing, the cells were stained with 7-AAD, anti-CD11c and CD8α or MHC class II and analyzed by four-color flow cytometry. The uptake of GFP-expressing bacteria by viable (7-AAD$^-$) CD11c$^+$CD8α^+, CD11c$^+$CD8α^-, CD11c$^+$ MHC class II^{low}, and CD11c$^+$ MHC class II^{high} cells is shown. The percentage of GFP$^+$ cells in the indicated DC population following incubation of liver DC with bacteria in the presence (dotted line, lower percentage) or absence (thick line, upper percentage) of CCD is indicated in each histogram. One representative experiment of four is shown.

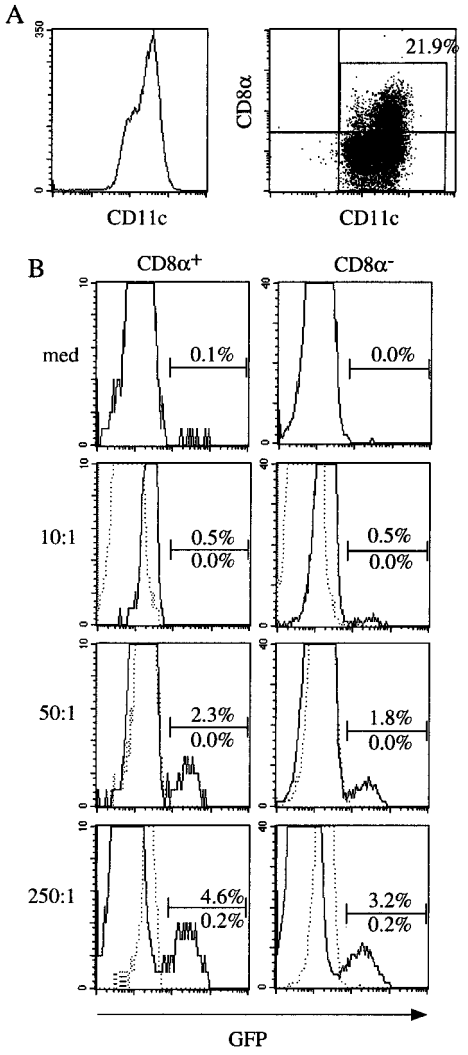

FIGURE 3. Uptake of *Salmonella* by liver DC from FL-injected mice. *A*, Purified, viable (7-AAD$^-$) liver DC from FL-injected mice were stained for surface expression of CD11c and CD8α and analyzed by flow cytometry. A dot plot of CD8α and CD11c expression on gated live (7-AAD$^-$) cells and histogram of CD11c expression are shown. *B*, Liver DC were incubated in medium alone (med) or with *S. typhimurium* χ4550/OVA-GFP for 2 h at the indicated bacteria to DC ratios. Cells were subsequently stained for CD11c and CD8α expression and GFP on gated, viable (7-AAD$^-$) CD11c$^+$CD8α^+ or CD11c$^+$CD8α^- cells is shown. The percentage of GFP$^+$ cells in the indicated DC population following incubation of liver DC with bacteria in the presence (dotted line, lower percentage) or absence (thick line, upper percentage) of CCD is indicated in each histogram. One representative experiment of four is shown.

cytometry difficult. Consistent with this, assessing bacterial numbers in DC by lysing purified CD11c$^+$ cells from these orally infected mice and plating on Luria-Bertani agar plates revealed that 0.03% of the DC contained bacteria. In addition, the inability to detect GFP$^+$ cells by flow cytometry was not due to loss of GFP, as bacteria recovered from sacrificed mice retained fluorescence. As the fraction of phagocytes containing bacteria is very low even in heavily infected moribund mice after oral *Salmonella* infection (50), an i.v. infection route was instead used to assess the capacity of liver DC to take up *Salmonella* in vivo. Analysis of purified DC from these mice revealed that ~0.3% of the liver DC had phagocytosed *Salmonella* 4 h after bacterial administration (Fig. 4). Thus, both CD8α^+ and CD8α^- liver DC are able to phagocytose *Salmonella* in vitro and in vivo. In addition, the uptake of GFP-expressing bacteria in vitro requires cytoskeletal rearrangements.

Liver DC can process and present Salmonella *Ags*

The capacity of freshly isolated liver DC to process and present bacterial Ags was investigated using liver DC from naive or FL-injected mice. Purified $OVA_{257–264}/K^b$-specific CD8$^+$ T cells from OT-I mice proliferated following a brief (2 h) coculture of liver DC with *S. typhimurium* expressing Crl-OVA (Fig. 5). The observed proliferation of OT-I cells was peptide-specific, as demonstrated by the lack of proliferation when *S. typhimurium* expressing Crl-HEL, which contains an epitope irrelevant for OT-I cells, was used (Fig. 5, *A–C*). Similarly, coculture of liver DC from naive or FL-injected BALB/c mice with *S. typhimurium* expressing OVA resulted in proliferation of $OVA_{323–339}/I\text{-}A^d$-specific DO11.10 T cells (Fig. 6). Proliferation of DO11.10 T cells was also epitope-specific, as proliferation was abrogated when *S. typhimurium* expressing Crl-OVA, which lacks the $OVA_{323–339}$ epitope recognized by DO11.10 cells, was used (Fig. 6*A*). Pretreatment of liver DC with CCD showed that active uptake of *Salmonella* was required for K^b presentation of $OVA_{257–264}$ from bacteria expressing

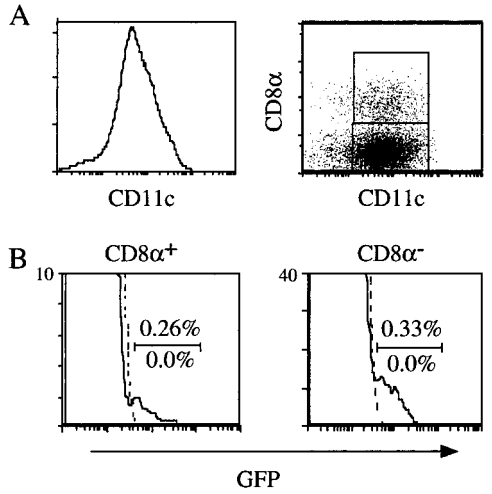

FIGURE 4. Uptake of *Salmonella* by liver DC in vivo. *A*, Purified, viable (7-AAD$^-$) liver DC from mice infected i.v. with χ4550/OVA-GFP or χ4550/OVA were stained for surface expression of CD11c and CD8α and analyzed by flow cytometry. A histogram of CD11c expression and a dot plot of CD8α and CD11c expression on gated live (7-AAD$^-$) cells are shown. *B*, GFP on gated, viable (7-AAD$^-$) CD11c$^+$CD8α^+ or CD11c$^+$CD8α^- cells is shown. The percentage of GFP$^+$ cells in the indicated DC population from the liver of mice infected with χ4550/OVA (dotted line, lower percentage) or χ4550/OVA-GFP (thick line, upper percentage) is indicated in each histogram. One representative experiment of three is shown.

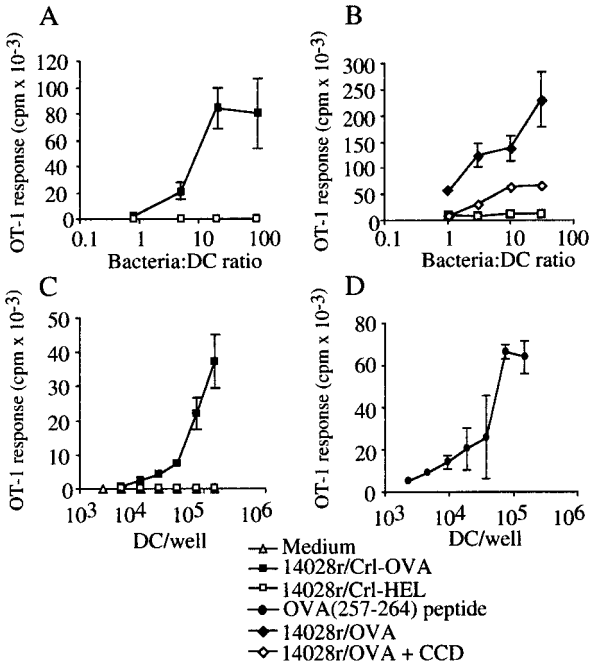

FIGURE 5. Freshly isolated, MACS-purified liver DC can process *Salmonella* for peptide presentation on MHC class I. *A*, Liver DC were coincubated at different bacteria to DC ratios with *S. typhimurium* 14028r expressing Crl-OVA (■), or as a control for epitope specificity, 14028r expressing Crl-HEL (□). *B*, Liver DC were coincubated with *S. typhimurium* 14028r expressing OVA either in the absence (◆) or presence (◇) of CCD to show the requirement for cytoskeletal rearrangements for presentation of the $OVA_{257–264}$ epitope from *Salmonella* expressing OVA. *A* and *B*, Bacteria were cocultured with DC for 2 h and the cells were washed, resuspended in medium containing 50 μg/ml gentamicin, and coincubated with MACS-purified (>95% pure) OT-I cells for 72 h. [^{3}H]Thymidine was added during the last 6–8 h. *C* and *D*, Liver DC were coincubated with *Salmonella* expressing Crl-OVA (■) or Crl-HEL (□) at a 25:1 bacteria to DC ratio or in medium alone (△) (*C*) or with 0.1 nM $OVA_{257–264}$ peptide (●) (*D*). Bacterial infection or incubation with peptide was for 2 h. DC were then washed, serially diluted 2-fold and OT-I proliferation was measured as in *A* and *B*. One representative experiment of three is shown. *A*, DC were purified from the liver of naive C57BL/6 mice. *B–D*, DC were purified from the liver of FL-injected C57BL/6 mice. In all cases, liver DC were >90% pure.

Crl-OVA (Fig. 5*B*) and for I-A^d presentation of $OVA_{323–339}$ from *Salmonella* expressing OVA (data not shown).

Cytokine production by liver DC after coculture with Salmonella

To investigate the cytokine production profile of liver DC that take up *Salmonella*, liver DC from FL-injected mice were coincubated with *Salmonella* expressing GFP, and intracellular cytokine production by gated CD11c$^+$CD8α^+ and CD11c$^+$CD8α^- cells was analyzed. Addition of bacteria to the cultures resulted in an increase in TNF-α-positive and IL-12p40$^+$ cells among CD8α^+ as well as CD8α^- liver DC (Fig. 7). However, only ~3.5% of IL-12p40$^+$ and 10% of TNF-α-positive cells contained bacteria (i.e., were GFP$^+$) in both of these DC subsets. This shows that a significant amount of cytokine production by noninfected DC occurs. Consistent with this, cytokine production was not abrogated when liver DC were pretreated with CCD (data not shown). Approximately 30 and 24% of GFP$^+$ DC produced TNF-α (i.e., were GFP$^+$ TNF-α-positive) for CD8α^+ and CD8α^- DC, respectively (Fig. 7). Likewise, ~46 and ~33% of GFP$^+$ cells stained positive for IL-12p40. Thus, uptake of *Salmonella* resulted in only a fraction of cells producing these cytokines.

In contrast to the capacity of liver DC to produce IL-12p40 and TNF-α following *Salmonella* encounter, no significant increase in IL-10$^+$ CD8α^- or CD8α^+ DC was detected by flow cytometry analysis after a 2 h pulse with bacteria (Fig. 7). Significant intracellular IL-10 in *Salmonella*-pulsed DC was not apparent despite detection of intracellular IL-10 by splenic CD4$^+$ T cells stimulated in the same experiments to produce this cytokine (according to Becton Dickinson's recommended protocol), which served as a positive control. In addition, IL-10 was not detected by ELISA after liver DC were pulsed with *Salmonella* for 2 h and cocultured for a total of 96 h either in the presence or absence of CD40L-expressing fibroblasts (data not shown). However, 4000 and 1000 pg/ml of IL-10 was detected in supernatants of bone marrow DC cultured together with zymosan (10 μg/ml; Molecular Probes, Leiden, The Netherlands) plus anti-CD40 (FGK45, 10 μg/ml) or LPS (*E. coli* 026:B6 LPS, 10 μg/ml; Sigma-Aldrich) plus anti-CD40, respectively, performed in parallel with the experiments using liver DC. The level of sensitivity of the IL-10 ELISA was 100 pg/ml. Thus, IL-12p40$^+$ and TNF-α-positive, but not IL-10-producing, CD8α^- and CD8α^+ liver DC are detected after a brief encounter with *S. typhimurium*.

Discussion

Liver DC have a role in oral tolerance as well as tumor and allograft rejection and may be involved in immune responses in the liver to hepatotropic bacteria (1, 51–54). The dichotomous behavior of liver DC in the immune system was the impetus for investigating the role of these cells in immunity to the hepatotropic intracellular bacterium *Salmonella*. The data show that freshly isolated liver DC phagocytose and process *Salmonella* for peptide presentation on MHC class I and class II to TCR transgenic T cells. In addition, liver DC were found to produce TNF-α and IL-12p40, but not IL-10, after a brief encounter with *Salmonella*.

The flow cytometry data presented in this study show that the vast majority of freshly isolated liver CD11c$^+$ cells express neither

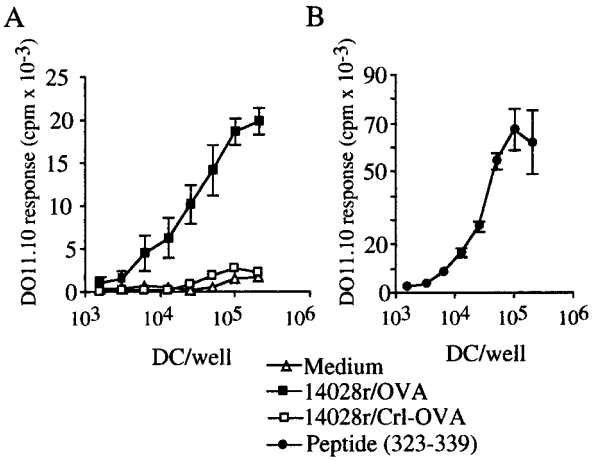

FIGURE 6. Freshly isolated, MACS-purified liver DC can process and present bacterial Ags on MHC class II to T cells. *A*, Liver DC were purified and incubated in medium only (△) or were cocultured with *Salmonella* at the bacteria to DC ratio of 10:1 with 14028r/OVA (■) or, as a control for epitope specificity, 14028r/Crl-OVA (□), which lacks the $OVA_{323–339}$ epitope. *B*, Liver DC were incubated with 1 μg/ml $OVA_{323–339}$ peptide (●). After washing, the cells were serially 2-fold diluted and MACS-purified DO11.10 T cells (≥95% pure) were added. The proliferative response was measured as in Fig. 5. DC were purified from the liver of FL-injected BALB/c mice and were at least 90% pure. One representative experiment of two is shown.

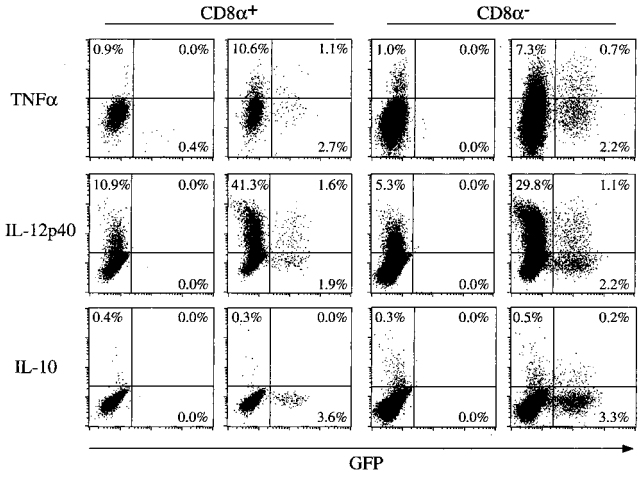

FIGURE 7. Cytokine production by liver DC after encountering *Salmonella*. Liver DC purified from FL-injected C57BL/6 mice were coincubated with χ4550/OVA-GFP for 2 h, washed, and treated with brefeldin A for 5 h. Cells were then stained for surface molecule expression and intracellular cytokines and were analyzed by four-color flow cytometry. Dot plots show bacterial association (GFP) and intracellular cytokine production by $CD11c^+CD8\alpha^+$ or $CD8\alpha^-$ live ($7\text{-}AAD^-$) liver DC. The percentage of cells within each quadrant is indicated. The *left* dot plot for $CD8\alpha^+$ and $CD8\alpha^-$ DC show background fluorescence without bacterial addition. One representative experiment of three is shown. Similar results were obtained when liver DC were purified from FL-injected BALB/c mice (data not shown).

$CD8\alpha$ nor CD4. This is in contrast to splenic DC, where ~50% of $CD11c^+$ cells are $CD8\alpha^-CD4^+$ and ~25% are $CD8\alpha^+CD4^-$ with the remaining fraction being $CD8\alpha^-CD4^-$ (9). A very small population of $CD4^+CD11c^+$ cells was present in the liver of naive mice, as also shown by Lian and colleagues (13). $CD11c^+$ cells from the liver of FL-injected mice had an even smaller fraction of $CD4^+$ cells than that in naive animals, whereas the percentage of $CD8^+$ cells among $CD11c^+$ cells increased. A similar relative reduction in the percentage of $CD4^+$ splenic DC has also been observed in the spleen of mice treated with recombinant human FL (Ref. 55 and our unpublished observations).

Among liver $CD11c^+CD8\alpha^-CD4^-$ cells, $CD11b^{high}$ and $CD11b^{low\text{-}int}$ populations were evident. These populations were somewhat more distinct when $CD11c^+$ cells from the liver of FL-injected mice were examined. This latter population of $CD11c^+$ cells ($CD8\alpha^-CD4^-CD11b^{low}$) is similar to DC subsets in PP and MLN and is not a major population in splenic DC (Ref. 10, 11 and Fig. 1*A*). Thus, the subset composition of liver DC shares some features with $CD11c^+$ cells in PP, which lack expression of CD4 (10), or with MLN DC, which have low expression of this molecule (9). This is in contrast to the spleen, in which the dominant DC subset expresses CD4 (9, 56). These data suggest that tissues draining the gut, the PP, MLN, and the liver, have a subset of DC that is only a minor component of DC in the spleen or other peripheral lymph nodes.

DC can be induced to undergo maturation by several signals including LPS and proinflammatory cytokines (49, 57). Surface expression of CD40 and CD86 on freshly isolated $CD11c^+$ cells from the liver of naive mice was relatively low and MHC class II expression was heterogeneous. These data suggest an immature phenotype of these cells (6). The data showing that freshly isolated liver DC can phagocytose *Salmonella* are consistent with an immature phenotype of the cells (6). This is further supported by data showing that overnight culture of freshly isolated $CD11c^+$ cells from liver resulted in a population of cells with higher, more uniform surface expression of MHC class II, CD40, and CD86 (Ref. 4 and our unpublished observations). Freshly isolated $CD11c^+$ liver cells from FL-injected mice had higher expression of CD86 than $CD11c^+$ liver cells from naive mice indicating that these DC may have a somewhat more mature phenotype.

Similar to data obtained using freshly isolated splenic DC (18), the capacity to phagocytose *Salmonella* after a brief coculture was found among both $CD8\alpha^+$ and $CD8\alpha^-$ liver DC. Moreover, this ability was much more apparent among MHC class II^{high} cells relative to MHC class II^{low} cells in the bulk $CD11c^+$ liver DC population. Liver DC with low MHC class II expression could possibly be DC progenitors and therefore not have the ability to phagocytose. A progenitor or very immature phenotype for this population is further supported by the disappearance of the MHC class II^{low} cells upon overnight culture (our unpublished observations). Moreover, the observation that freshly isolated liver DC can process *Salmonella* for peptide presentation on MHC class I and class II, a capacity that is down-regulated upon DC maturation (6, 49, 58, 59), further support an immature phenotype of liver DC.

Freshly isolated liver DC also respond to *Salmonella* infection by producing cytokines. For example, IL-$12p40^+$ and TNF-α-positive cells were found among $CD8\alpha^+$ and $CD8\alpha^-$ liver DC after a brief coculture with *Salmonella*. A somewhat higher fraction of $CD8\alpha^+$ compared with $CD8\alpha^-$ DC in the liver were IL-$12p40^+$ following *Salmonella* encounter, but both subsets had the capacity to produce this cytokine upon bacterial exposure. Moreover, DC (MACS-purified $CD11c^+$ cells) from the liver of FL-injected mice pulsed with *Salmonella* for 2 h followed by washing and continued culture in the presence of CD40L-transfected fibroblasts produced IL-12p70 (detected by ELISA; data not shown). In contrast, IL-12p70 was not detected in supernatants in which mock-transfected fibroblasts were substituted for CD40L transfectants despite that IL-12p40 was detected in both culture conditions. These results are similar to those showing that CD40 engagement greatly augments the capacity of splenic DC to produce IL-12p70 upon microbial

stimulus (22). Thus, liver DC appear to have the capacity to produce the biologically active form of IL-12 upon receiving synergistic signals from bacterial encounter and CD40 engagement.

For TNF-α production by liver DC upon *Salmonella* encounter, no clear distinction was found among the capacity of hepatic DC subsets to produce TNF-α in the conditions tested. In addition, we were unable to detect IL-10 by flow cytometry after *Salmonella* encounter or by ELISA performed on culture supernatants of liver DC pulsed with *Salmonella* and cultured for up to 96 h in the presence of either CD40L- or mock-transfected fibroblasts. Thus, we did not detect IL-10 production by freshly isolated liver DC after *Salmonella* encounter despite that liver DC progenitors have been suggested to secrete IL-10 in MLR cultures (29). Purified hepatic DC exposed to *Salmonella* ex vivo produce a similar cytokine profile as have previously been shown for splenic DC upon *Salmonella* encounter (18). However, other factors such as the type of microbial stimulus, cytokines, cognate interactions, and the genotype of *Salmonella* can influence the cytokine production capacity of DC (21, 22, 60, 61), and hepatic DC may be influenced by the microenvironment in the liver and thereby possibly produce a different cytokine profile in situ.

Thus, DC in the liver have the capacity to process and present *Salmonella* Ags and produce cytokines important in host defense against this bacterium (62). These data together with the observation that *Salmonella*-specific T cells are found in the liver after oral *Salmonella* infection (A. C. Kirby and M. J. Wick, manuscript in preparation) suggest a potential role of hepatic DC in the immune response to oral infection with hepatotropic bacteria. In addition, liver DC harbor *Salmonella* during infection, as has been shown for splenic DC (18, 39). However, whether resident liver DC internalize bacteria present in the blood or whether intestinal DC take up orally acquired bacteria and migrate into deeper tissues such as the liver remains to be determined (37, 38, 63). Despite the coexistence of DC with the capacity to process and present *Salmonella* Ags and *Salmonella*-specific T cells in the liver, the site at which hepatic T cells are primed and the role of liver DC in this process during the immune response to orally acquired bacteria remains to be established.

Acknowledgments

We are grateful to Allan Mowat, Glasgow University, Glasgow, Scotland, for providing OT-I mice and to Susanna Grundström, Active Biotech, Lund, Sweden, for DO11.10 mice.

References

1. Wick, M. J., F. Leithauser, and J. Reimann. 2002. The hepatic immune system. *Crit. Rev. Immunol. 22:47*.
2. Woo, J., L. Lu, A. S. Rao, Y. Li, V. Subbotin, T. E. Starzl, and A. W. Thomson. 1994. Isolation, phenotype, and allostimulatory activity of mouse liver dendritic cells. *Transplantation 58:484*.
3. Limmer, A., J. Ohl, C. Kurts, H. G. Ljunggren, Y. Reiss, M. Groettrup, F. Momburg, B. Arnold, and P. A. Knolle. 2000. Efficient presentation of exogenous antigen by liver endothelial cells to $CD8^+$ T cells results in antigen-specific T-cell tolerance. *Nat. Med. 6:1348*.
4. O'Connell, P. J., A. E. Morelli, A. J. Logar, and A. W. Thomson. 2000. Phenotypic and functional characterization of mouse hepatic $CD8\alpha^+$ lymphoid-related dendritic cells. *J. Immunol. 165:795*.
5. Bertolino, P., M. C. Trescol-Biemont, and C. Rabourdin-Combe. 1998. Hepatocytes induce functional activation of naive $CD8^+$ T lymphocytes but fail to promote survival. *Eur. J. Immunol. 28:221*.
6. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245*.
7. Matsuno, K., S. Kudo, and T. Ezaki. 1997. The liver sinusoids as a specialized site for blood-lymph translocation of rat dendritic cells. *Adv. Exp. Med. Biol. 417:77*.
8. Kudo, S., K. Matsuno, T. Ezaki, and M. Ogawa. 1997. A novel migration pathway for rat dendritic cells from the blood: hepatic sinusoids-lymph translocation. *J. Exp. Med. 185:777*.
9. Shortman, K., and Y. J. Liu. 2002. Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol. 2:151*.
10. Iwasaki, A., and B. L. Kelsall. 2001. Unique functions of $CD11b^+$, $CD8\alpha^+$, and double-negative Peyer's patch dendritic cells. *J. Immunol. 166:4884*.
11. Iwasaki, A., and B. L. Kelsall. 2000. Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3α, MIP-3β, and secondary lymphoid organ chemokine. *J. Exp. Med. 191:1381*.
12. Trobonjaca, Z., F. Leithauser, P. Moller, R. Schirmbeck, and J. Reimann. 2001. Activating immunity in the liver. I. Liver dendritic cells (but not hepatocytes) are potent activators of IFN-γ release by liver NKT cells. *J. Immunol. 167:1413*.
13. Lian, Z.-X., T. Okada, X.-S. He, H. Kita, Y.-J. Liu, A. A. Ansari, K. Kikuchi, S. Ikehara, and M. E. Gershwin. 2003. Heterogeneity of dendritic cells in the mouse liver: identification and characterization of four distinct populations. *J. Immunol. 170:2323*.
14. Valdez, Y., W. Mah, M. M. Winslow, L. Xu, P. Ling, and S. E. Townsend. 2002. Major histocompatibility complex class II presentation of cell-associated antigen is mediated by $CD8\alpha^+$ dendritic cells in vivo. *J. Exp. Med. 195:683*.
15. Iyoda, T., S. Shimoyama, K. Liu, Y. Omatsu, Y. Akiyama, Y. Maeda, K. Takahara, R. M. Steinman, and K. Inaba. 2002. The $CD8^+$ dendritic cell subset selectively endocytoses dying cells in culture and in vivo. *J. Exp. Med. 195:1289*.
16. Hochrein, H., K. Shortman, D. Vremec, B. Scott, P. Hertzog, and M. O'Keeffe. 2001. Differential production of IL-12, IFN-α, and IFN-γ by mouse dendritic cell subsets. *J. Immunol. 166:5448*.
17. Reis e Sousa, C., G. Yap, O. Schulz, N. Rogers, M. Schito, J. Aliberti, S. Hieny, and A. Sher. 1999. Paralysis of dendritic cell IL-12 production by microbial products prevents infection-induced immunopathology. *Immunity 11:637*.
18. Yrlid, U., and M. J. Wick. 2002. Antigen presentation capacity and cytokine production by murine splenic dendritic cell subsets upon *Salmonella* encounter. *J. Immunol. 169:108*.
19. Pooley, J. L., W. R. Heath, and K. Shortman. 2001. Cutting edge: intravenous soluble antigen is presented to CD4 T cells by $CD8^-$ dendritic cells, but cross-presented to CD8 T cells by $CD8^+$ dendritic cells. *J. Immunol. 166:5327*.
20. Maldonado-Lopez, R., T. De Smedt, P. Michel, J. Godfroid, B. Pajak, C. Heirman, K. Thielemans, O. Leo, J. Urbain, and M. Moser. 1999. $CD8\alpha^+$ and $CD8\alpha^-$ subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J. Exp. Med. 189:587*.
21. Maldonado-Lopez, R., C. Maliszewski, J. Urbain, and M. Moser. 2001. Cytokines regulate the capacity of $CD8\alpha^+$ and $CD8\alpha^-$ dendritic cells to prime Th1/Th2 cells in vivo. *J. Immunol. 167:4345*.
22. Schulz, O., D. A. Edwards, M. Schito, J. Aliberti, S. Manickasingham, A. Sher, and C. Reis e Sousa. 2000. CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells in vivo requires a microbial priming signal. *Immunity 13:453*.
23. Reis e Sousa, C., S. Hieny, T. Scharton-Kersten, D. Jankovic, H. Charest, R. N. Germain, and A. Sher. 1997. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J. Exp. Med. 186:1819*.
24. Pulendran, B., P. Kumar, C. W. Cutler, M. Mohamadzadeh, T. Van Dyke, and J. Banchereau. 2001. Lipopolysaccharides from distinct pathogens induce different classes of immune responses in vivo. *J. Immunol. 167:5067*.
25. Huang, L. Y., C. Reis e Sousa, Y. Itoh, J. Inman, and D. E. Scott. 2001. IL-12 induction by a TH1-inducing adjuvant in vivo: dendritic cell subsets and regulation by IL-10. *J. Immunol. 167:1423*.
26. Liu, Y. J., H. Kanzler, V. Soumelis, and M. Gilliet. 2001. Dendritic cell lineage, plasticity and cross-regulation. *Nat. Immun*ol. *2:585*.
27. den Haan, J. M., and M. J. Bevan. 2002. Constitutive versus activation-dependent cross-presentation of immune complexes by $CD8^+$ and $CD8^-$ dendritic cells in vivo. *J. Exp. Med. 196:817*.
28. Iwasaki, A., and B. L. Kelsall. 1999. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J. Exp. Med. 190:229*.
29. Khanna, A., A. E. Morelli, C. Zhong, T. Takayama, L. Lu, and A. W. Thomson. 2000. Effects of liver-derived dendritic cell progenitors on Th1- and Th2-like cytokine responses in vitro and in vivo. *J. Immunol. 164:1346*.
30. Knodler, L. A., and B. B. FIndlay. 2001. *Salmonella* and apoptosis: to live or let die? *Microb. Infect. 3:1321*.
31. Richter-Dahlfors, A., A. M. J. Buchan, and B. B. Finlay. 1997. Murine salmonellosis studied by confocal microscopy: *Salmonella typhimurium* resides intracellularly inside macrophages and exerts a cytotoxic effect on phagocytes in vivo. *J. Exp. Med. 186:569*.
32. Yrlid, U., and M. J. Wick. 2000. *Salmonella*-induced apoptosis of infected macrophages results in presentation of a bacteria-encoded antigen after uptake by bystander dendritic cells. *J. Exp. Med. 191:613*.
33. Monack, D. M., D. Hersh, N. Ghori, D. Bouley, A. Zychlinsky, and S. Falkow. 2000. *Salmonella* exploits caspase-1 to colonize Peyer's patches in a murine typhoid model. *J. Exp. Med. 192:249*.
34. Ohl, M. E., and S. I. Miller. 2001. *Salmonella*: a model for bacterial pathogenesis. *Annu. Rev. Med. 52:259*.
35. Wijburg, O. L. C., N. van Rooijen, and R. A. Strugnell. 2002. Induction of $CD8^+$ T lymphocytes by *Salmonella typhimurium* is independent of *Salmonella* pathogenicity island 1-mediated host cell death. *J. Immunol. 169:3275*.
36. Sukupolvi, S., A. Edelstein, M. Rhen, S. J. Normark, and J. D. Pfeifer. 1997. Development of a murine model of chronic *Salmonella* infection. *Infect. Immun. 65:838*.

37. Vazquez-Torres, A., J. Jones-Carson, A. J. Baumler, S. Falkow, R. Valdivia, W. Brown, M. Le, R. Berggren, W. T. Parks, and F. C. Fang. 1999. Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. *Nature 401:804*.
38. Hopkins, S. A., F. Niedergang, I. E. Corthesy-Theulaz, and J. P. Kraehenbuhl. 2000. A recombinant *Salmonella typhimurium* vaccine strain is taken up and survives within murine Peyer's patch dendritic cells. *Cell. Microbiol. 2:59*.
39. Yrlid, U., M. Svensson, A. Hakansson, B. J. Chambers, H. G. Ljunggren, and M. J. Wick. 2001. In vivo activation of dendritic cells and T cells during *Salmonella enterica* serovar Typhimurium infection. *Infect. Immun. 69:5726*.
40. Sundquist, M., A. Rydström, and M. J. Wick. 2004. Immunity to *Salmonella* from a dendritic point of view. *Cell. Microbiol. 6:1*.
41. Masopust, D., V. Vezys, A. L. Marzo, and L. Lefrancois. 2001. Preferential localization of effector memory cells in nonlymphoid tissue. *Science 291:2413*.
42. Pope, C., S. K. Kim, A. Marzo, D. Masopust, K. Williams, J. Jiang, H. Shen, and L. Lefrancois. 2001. Organ-specific regulation of the CD8 T cell response to *Listeria monocytogenes* infection. *J. Immunol. 166:3402*.
43. Kursar, M., K. Bonhagen, A. Kohler, T. Kamradt, S. H. Kaufmann, and H. W. Mittrucker. 2002. Organ-specific $CD4^+$ T cell response during *Listeria monocytogenes* infection. *J. Immunol. 168:6382*.
44. Hogquist, K. A., S. C. Jameson, W. R. Heath, J. L. Howard, M. J. Bevan, and F. R. Carbone. 1994. T cell receptor antagonist peptides induce positive selection. *Cell 76:17*.
45. Hsieh, C. S., S. E. Macatonia, A. O'Garra, and K. M. Murphy. 1995. T cell genetic background determines default T helper phenotype development in vitro. *J. Exp. Med. 181:713*.
46. Svensson, M., B. Stockinger, and M. J. Wick. 1997. Bone marrow-derived dendritic cells can process bacteria for MHC-I and MHC-II presentation to T cells. *J. Immunol. 158:4229*.
47. Wick, M. J., and J. D. Pfeifer. 1996. MHC-I presentation of $OVA_{257–264}$ from exogenous sources: protein context influences the degree of TAP-independent presentation. *Eur. J. Immunol. 26:2790*.
48. Nakayama, K., S. M. Kelly, and R. Curtiss III. 1988. Construction of an ASD^+ expression-cloning vector: stable maintenance and high level expression of cloned genes in a *Salmonella* vaccine strain. *Bio/Technology 6:693*.
49. Svensson, M., C. Johansson, and M. J. Wick. 2000. *Salmonella enterica* serovar Typhimurium-induced maturation of bone marrow-derived dendritic cells. *Infect. Immun. 68:6311*.
50. Salcedo, S. P., M. Noursadeghi, J. Cohen, and D. W. Holden. 2001. Intracellular replication of *Salmonella typhimurium* strains in specific subsets of splenic macrophages in vivo. *Cell Microbiol. 3:587*.
51. Lau, A. H., and A. W. Thomson. 2003. Dendritic cells and immune regulation in the liver. *Gut 52:307*.
52. Thomson, A. W., M. L. Drakes, A. F. Zahorchak, P. J. O'Connell, R. J. Steptoe, S. Qian, and L. Lu. 1999. Hepatic dendritic cells: immunobiology and role in liver transplantation. *J. Leukocyte Biol. 66:322*.
53. Viney, J. L., A. M. Mowat, J. M. O'Malley, E. Williamson, and N. A. Fanger. 1998. Expanding dendritic cells in vivo enhances the induction of oral tolerance. *J. Immunol. 160:5815*.
54. Yoneyama, H., K. Matsuno, Y. Zhang, M. Murai, M. Itakura, S. Ishikawa, G. Hasegawa, M. Naito, H. Asakura, and K. Matsushima. 2001. Regulation by chemokines of circulating dendritic cell precursors, and the formation of portal tract-associated lymphoid tissue, in a granulomatous liver disease. *J. Exp. Med. 193:35*.
55. O'Keeffe, M., H. Hochrein, D. Vremec, J. Pooley, R. Evans, S. Woulfe, and K. Shortman. 2002. Effects of administration of progenipoietin 1, Flt-3 ligand, granulocyte colony-stimulating factor, and pegylated granulocyte-macrophage colony-stimulating factor on dendritic cell subsets in mice. *Blood 99:2122*.
56. Vremec, D., J. Pooley, H. Hochrein, L. Wu, and K. Shortman. 2000. CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. *J. Immunol. 164:2978*.
57. Winzler, C., P. Rovere, M. Rescigno, F. Granucci, G. Penna, L. Adorini, V. S. Zimmermann, J. Davoust, and P. Ricciardi-Castagnoli. 1997. Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. *J. Exp. Med. 185:317*.
58. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: Downregulation by cytokines and bacterial products. *J. Exp. Med. 182:389*.
59. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulation by tumor necrosis factor α. *J. Exp. Med. 179:1109*.
60. Edwards, A. D., S. P. Manickasingham, R. Spörri, S. S. Diebold, O. Schultze, A. Sher, T. Kaiso, S. Akira, and C. Reis e Sousa. 2002. Microbial recognition via Toll-like receptor-dependent and -independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. *J. Immunol. 169:3652*.
61. Dreher, D., M. Kok, L. Cochand, S. G. Kiama, P. Gehr, J.-C. Perchère, and L. P. Nicod. 2001. Genetic background of attenuated *Salmonella typhimurium* has profound influence on infection and cytokine patterns in human dendritic cells. *J. Leukocyte Biol. 69:583*.
62. Mittrucker, H. W., and S. H. Kaufmann. 2000. Immune response to infection with *Salmonella typhimurium* in mice. *J. Leukocyte Biol. 67:457*.
63. Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl, and P. Ricciardi-Castagnoli. 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immun. 2:361*.