Monocyte-Mediated Inhibition of TLR9-Dependent IFN-α Induction in Plasmacytoid Dendritic Cells Questions Bacterial DNA as the Active Ingredient of Bacterial Lysates

Jens M. Poth, Christoph Coch, Nicolas Busch, Olaf Boehm, Martin Schlee, Markus Janke, Thomas Zillinger, Oliver Schildgen, Winfried Barchet and Gunther Hartmann

J Immunol published online 5 November 2010
http://www.jimmunol.org/content/early/2010/11/05/jimmunol.1001798

Supplementary Material
http://www.jimmunol.org/content/suppl/2010/11/05/jimmunol.1001798.DC1

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
Monocyte-Mediated Inhibition of TLR9-Dependent IFN-α Induction in Plasmacytoid Dendritic Cells Questions Bacterial DNA as the Active Ingredient of Bacterial Lysates

Jens M. Poth,* Christoph Coch,* Nicolas Busch,* Olaf Boehm,† Martin Schlee,* Markus Janke,* Thomas Zillinger,* Oliver Schildgen,‡ Winfried Barchet,* and Gunther Hartmann*†

Bacterial DNA contains unmethylated CpG dinucleotides and is a potent ligand for TLR9. Bacterial DNA has been claimed the active ingredient in bacterial lysates used for immunotherapy. Whereas the detection of viral DNA by TLR9 expressed in plasmacytoid dendritic cells (PDCs) with subsequent IFN-α production is well defined, the role of bacterial DNA during microbial infection is less clear. In fact, IFN-α is not a hallmark of antibacterial immune responses. Unlike in mice, TLR9 expression in humans is restricted to PDCs and B cells; thus, conclusions from murine models of infection have limitations. In this study, we demonstrate that lysates of heat-killed Escherichia coli containing bacterial DNA induced IFN-α in isolated PDCs but not in the mixed cell populations of human PBMCs. Depletion of monocytes restored IFN-α secretion by PDCs within PBMCs. We found that monocyte-derived IL-10 and PGs contribute to monocyte-mediated inhibition of IFN-α release in PDCs. We conclude that human PDCs can be stimulated by bacterial DNA via TLR9; however, in the physiological context of mixed-cell populations, PDC activation is blocked by factors released from monocytes stimulated in parallel by other components of bacterial lysates such as LPS. This functional repression of PDCs by concomitantly stimulated monocytes avoids production of antiviral IFN-α during bacterial infection and thus explains how the innate immune system is enabled to distinguish bacterial from viral CpG DNA and thus to elicit the appropriate responses despite the presence of CpG DNA in both types of infection. The Journal of Immunology, 2010, 185: 000–000.

The online version of this article contains supplemental material.

Abbreviations used in this paper: BCG, bacillus Calmette-Guérin; Control, PBS; CQ, chloroquine; bARE, heat-killed Escherichia coli; MSN, isolated monocytes stimulated with heat-killed Escherichia coli; MSN-2h, supernatants of isolated monocytes stimulated with heat-killed Escherichia coli for 2 h; MSN-F, filtered supernatants of isolated monocytes stimulated with heat-killed Escherichia coli; MSN-HI, heat-inactivated supernatants of isolated monocytes stimulated with heat-killed Escherichia coli; MSN-HII, heat-inactivated supernatants of isolated monocytes incubated with indomethacin pretreatment with heat-killed Escherichia coli; MSN-I, isolated monocytes incubated with indomethacin pretreatment with heat-killed Escherichia coli; ND, not detectable; ODN, oligodeoxynucleotide; PDC, plasmacytid dendritic cell.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10/$16.00
fierently despite the presence of the same molecular pattern CpG DNA in both bacterial and viral infection.

In this study, we found that bacterial lysates containing CpG DNA induced IFN-α in purified PDCs but not if monocytes were present and that concomitant activation of monocytes by other constituents of bacterial lysates leads to the release of factors like PGNs and IL-10 that actively suppress IFN-α production in PDCs. As a consequence, CpG DNA in the context of other bacterial molecules is functionally ignored by PDCs, whereas CpG DNA in the absence of bacterial molecules induces IFN-α in PDCs; thus, CpG DNA represents a molecular pattern that represents viral infection rather than bacterial infection, and due to this functional deficit in bacterial infection, bacterial DNA may not be the active ingredient in bacterial lysates responsible for type I IFN-dependent antitumor activity.

Materials and Methods

Media and reagents

RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 2% AB-serum (Lonza, Basel, Switzerland) or 3% heat-inactivated FCS (Invitrogen), 1.5 mM L-glutamine (Cambrex, East Rutherford, NJ), 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Sigma-Aldrich, St. Louis, MO) was used. rIL-10, rTNF, and corresponding Abs were purchased from BD Pharmingen (San Diego, CA). The CpG oligodeoxynucleotide (ODN) 2336 [equivalent to ODN TTAGGTTGGGTGGGCTG (23)] was purchased from Invivogen (San Diego, CA). LPS, peptidoglycan, caffeine, indomethacin, and chloroquine (CQ) were purchased from Sigma-Aldrich. Z-YVAD was from Immunochimetry Technologies (Bloomington, MN). The CellTiter-Blue cell viability assay was from Promega (Madison, WI).

Cell culture and separation

Freshly prepared buffy coats from human donors were obtained from the Institute for Experimental Hematology and Transfusion Medicine, University Hospital of Bonn, Bonn, Germany with the donors’ written informed consent. PBMCs were prepared by density gradient centrifugation using Ficoll separating solution (Biochrom, Cambridge, U.K.) and lysis of RBCs using BD Pharm Lyse (BD Pharmingen) if necessary. PDCs and monocytes were selected from PBMCs using CD304 or CD14 microbeads (Miltenyi Biotec, Auburn, CA) and two separation columns according to the manufacturer’s instructions. Cell viability exceeded 95% as examined by trypan blue staining. Purity of PDCs was ≥95%. A total of 4 × 10^5 PDCs per well were cultured in 96-well plates (final volume 200 μl) unless stated otherwise.

Preparation of bacteria and virus

Escherichia coli K12 was grown overnight in standard Luria-Bertani medium, which was then replaced by 0.9% saline (B. Braun, Bethlehem, PA). Bacteria were adjusted to 1 × 10^8/ml using the OD600 and heat-killed at 80°C for 20 min (heat-killed E. coli [hkEC]). For digestion experiments, hkEC were incubated with the indicated concentrations of DNAse-I (Fermentas, Glen Burnie, MD) at 37°C for 30 min prestimulation. When cells were stimulated with HSV-1 17 syn+ (24), 3% (v/v) FCS (Invitrogen) was used instead of human AB serum.

In vitro cell stimulation

Cells were stimulated with 5 μg/ml CpG ODN. HSV-1 and pattern recognition receptor ligands were used at stated concentrations. When using neutralizing Abs (30 μg/ml, unless stated otherwise), CQ (concentrations as stated), z-YVAD (25 μM), indomethacin (1 mM), or caffeine (3 mM), cells were preincubated for 20–30 min with these reagents. According to the manufacturer’s protocol, cells were pretreated with ODN TTAGGG (25 μg/ml) for 1 h before the addition of hkEC. Unless where indicated otherwise, hkEC was used at a concentration of 10% (v/v).

Preparation of monocYTE-derived supernatants

Monocyte-derived supernatants were prepared by stimulating 1.6 × 10^6 monocytes with hkEC for 20 h (MSN-1) or 2 h. In some experiments, monocytes were preincubated with indomethacin before hkEC was added (MSN-2). MSN-1 was heat-inactivated by applying 90°C for 2 min for some experiments. In other experiments, MSN was filtered using a 20-μm filter or only heat-inactivated.

Cytokine analysis

Supernatants were harvested after 20 h and assayed for secreted cytokines by ELISA according to the manufacturers’ protocols (IFN-α module set from Bender MedSystems, Vienna, Austria; respective OptEIA kits [BD Pharmingen] for IL-12p70; IL-10, IL-6, IL-1β, and TNF).

Flow cytometry

Cell purity was assessed by FACS analysis of cells stained for CD14 (monocytes) and CD303 (PDCs) using an LSR II cytometer (BD Biosciences, San Jose, CA). Abs were from BD Biosciences (CD14-allophycocyanin) and Miltenyi Biotec (CD303-FITC) and used according to the manufacturers’ protocols. Data analysis was performed on viable cells (propidium iodide staining) postexclusion of doublets using FACSDiva software (BD Biosciences).

Statistical analysis

Data are expressed as means ± SEM. In some analyses, absolute cytokine concentrations were normalized to the appropriate positive control (100%). Statistical analyses were performed using Prism 5 software (GraphPad, San Diego, CA). Statistical significance of differences was determined by the unpaired two-tailed Student t test unless stated otherwise. Differences were considered statistically significant for p ≤ 0.05. In all figures, *p ≤ 0.05 and **p ≤ 0.01.

Results

Bacterial lysate induces IFN-α in purified PDCs but not in PBMCs

IFN-α is a marker cytokine for an antiviral innate immune response. Within human PBMCs, TLR9 is expressed in B cells and PDCs, but only PDCs produce IFN-α upon stimulation with CpG DNA. CpG DNA is contained in bacterial lysates, but little IFN-α is induced during bacterial infection. We compared human PBMCs and human isolated PDCs for their ability to produce IFN-α upon stimulation with CpG ODN 2336, HSV-1, and bacterial lysates. IFN-α induction by HSV-1 in PDCs was in the same range as CpG ODN and reached ~30% of IFN-α induction by CpG ODN in isolated PDCs (Fig. 1A). Opposite results were obtained with bacterial lysates (hkEC). Bacterial lysate induced considerable amounts of IFN-α in PDCs (up to 20% of IFN-α induced by CpG ODN 2336), but only little IFN-α in PBMCs (Fig. 1B). Thus, bacterial lysate is able to induce IFN-α in PDCs (purity degree ≥95%; Fig. 1C), but in the context of PBMCs, bacterial lysate-stimulated induction of IFN-α in PDCs seems suppressed, whereas this is not the case for CpG DNA and virus.

Bacterial lysate-induced IFN-α in human PDCs is DNA dependent

Bacterial lysate-induced IFN-α in PDCs purified from PBMCs was concentration dependent with a maximum at 10% (v/v) (Fig. 2A). This amount of hkEC did not alter cell viability (Supplemental Fig. 1) and was used for subsequent experiments. E. coli contain ligands for multiple innate immune receptors including TLR2 [bacterial lipoproteins (25)], TLR4 [LPS (26, 27)], TLR5 [flagellin (1, 28)], TLR7 [RNA (29)], and TLR9 [DNA (1, 8)]. However, PDCs exclusively express TLR7 and TLR9, which have a role in the recognition of specific bacterial products (26, 27). TLR9 ligands are CpG DNA and virus.

Cytokine analysis

Supernatants were harvested after 20 h and assayed for secreted cytokines by ELISA according to the manufacturers’ protocols (IFN-α module set from Bender MedSystems, Vienna, Austria; respective OptEIA kits [BD Pharmingen] for IL-12p70; IL-10, IL-6, IL-1β, and TNF).

Flow cytometry

Cell purity was assessed by FACS analysis of cells stained for CD14 (monocytes) and CD303 (PDCs) using an LSR II cytometer (BD Biosciences, San Jose, CA). Abs were from BD Biosciences (CD14-allophycocyanin) and Miltenyi Biotec (CD303-FITC) and used according to the manufacturers’ protocols. Data analysis was performed on viable cells (propidium iodide staining) postexclusion of doublets using FACSDiva software (BD Biosciences).

Statistical analysis

Data are expressed as means ± SEM. In some analyses, absolute cytokine concentrations were normalized to the appropriate positive control (100%). Statistical analyses were performed using Prism 5 software (GraphPad, San Diego, CA). Statistical significance of differences was determined by the unpaired two-tailed Student t test unless stated otherwise. Differences were considered statistically significant for p ≤ 0.05. In all figures, *p ≤ 0.05 and **p ≤ 0.01.

Results

Bacterial lysate induces IFN-α in purified PDCs but not in PBMCs

IFN-α is a marker cytokine for an antiviral innate immune response. Within human PBMCs, TLR9 is expressed in B cells and PDCs, but only PDCs produce IFN-α upon stimulation with CpG DNA. CpG DNA is contained in bacterial lysates, but little IFN-α is induced during bacterial infection. We compared human PBMCs and human isolated PDCs for their ability to produce IFN-α upon stimulation with CpG ODN 2336, HSV-1, and bacterial lysates. IFN-α induction by HSV-1 in PDCs was in the same range as CpG ODN and reached ~30% of IFN-α induction by CpG ODN in isolated PDCs (Fig. 1A). Opposite results were obtained with bacterial lysates (hkEC). Bacterial lysate induced considerable amounts of IFN-α in PDCs (up to 20% of IFN-α induced by CpG ODN 2336), but only little IFN-α in PBMCs (Fig. 1B). Thus, bacterial lysate is able to induce IFN-α in PDCs (purity degree ≥95%; Fig. 1C), but in the context of PBMCs, bacterial lysate-stimulated induction of IFN-α in PDCs seems suppressed, whereas this is not the case for CpG DNA and virus.
Finally, preincubation of PDCs with the inhibitory ODN TTAGGG, which has been shown to block TLR9-dependent cell activation (23), inhibited the hkEC induced IFN-α response in a dose-dependent fashion (Fig. 2D). Together, these data suggest that DNA within the bacterial lysate is recognized via endosomal TLR9 and is responsible for IFN-α induction in PDCs.

**FIGURE 1.** Bacterial lysate induces IFN-α in purified PDCs but not in PBMCs. Primary human PBMCs or purified PDCs were stimulated with CpG ODN 2336 (5 μg/ml) or with HSV-1 or hkEC as indicated. IFN-α was analyzed in the supernatants. A, PBMCs or PDCs were stimulated with CpG ODN or with increasing concentrations of HSV-1. B, PBMCs or PDCs were stimulated with CpG ODN 2336 or with increasing concentrations of hkEC. C, Purity of PDCs (CD14−CD303+). Data show the means ± SEM of three independent experiments (A, B) or one representative of three independent experiments (C).

**FIGURE 2.** Bacterial lysate-induced IFN-α induction in human PDCs is mediated by bacterial DNA and is TLR9 dependent. Human primary PDCs were incubated in the presence of hkEC. IFN-α was analyzed in the supernatants. A, IFN-α induction by increasing concentrations of hkEC. B, PDCs were stimulated with hkEC (10%, v/v) or with hkEC digested with DNase I (IFN-α by hkEC without DNase I, 100%: 942 ± 474 pg/ml). C, PDCs were stimulated with hkEC (10%, v/v), and endosomal maturation was inhibited by CQ. D, PDCs were stimulated with hkEC (10%, v/v) with or without the inhibitory ODN TTAGGG. Data are means ± SEM of three (A, C) or two (B) independent experiments. D shows means ± SEM of one of two independent experiments. *p < 0.05; **p < 0.01.
In PBMCs, TLR2/4-activated monocytes suppress bacterial lysate-induced TLR9-mediated IFN-α production in PDCs

Because bacterial lysate induced IFN-α in PDCs purified from PBMCs, but not in PBMCs, we hypothesized that bacterial lysate may activate another immune cell subset within PBMCs, leading to an inhibition of PDC function. For example, TLR2 and TLR4 ligands contained in bacterial lysate are able to stimulate monocytes. We found that simultaneous stimulation of PBMCs by CpG ODN 2336 together with a TLR2 ligand (proteoglycan) or a TLR4 ligand (LPS) strongly inhibited IFN-α induction compared with stimulation with CpG ODN 2336 alone (Fig. 3). Inhibition was further enhanced when TLR2 and TLR4 ligands were combined. To test whether monocytes contribute to inhibition of IFN-α induction within PBMCs, monocytes were depleted. The induction of IFN-α in PBMCs by bacterial lysate was restored in the absence of monocytes (Fig. 4A). Furthermore, IFN-α induction in isolated PDCs by bacterial lysate was suppressed when increasing numbers of purified monocytes were added (Fig. 4B) up to the physiological ratio of PDCs to monocytes in PBMCs (1:20; Fig. 4D, Table 1). Similar suppression of IFN-α in PDCs was seen when PDCs were stimulated in the presence of supernatants derived from monocytes incubated in the presence of bacterial lysate (Fig. 4C). Together, these data indicated that TLR2/4-activated monocytes in PBMCs are responsible for suppression of PDC-derived IFN-α in PBMCs exposed to bacterial lysate.

IL-10 and cyclooxygenase activity inhibit CpG DNA-induced IFN-α

Monocytes release the inhibitory cytokine IL-10 upon stimulation with bacteria-derived TLR ligands. We found that in PBMC stimulation with bacterial lysate but not with CpG, ODN induced high amounts of IL-10; depletion of monocytes from PBMCs revealed that most of IL-10 was secreted by monocytes (Fig. 5A). When rIL-10 was added to PDCs at similar quantities as detected in bacterial lysate-stimulated PBMC supernatants, IFN-α induction by either CpG ODN (Fig. 5B) or hKEC (Fig. 5C) was completely suppressed. Moreover, an anti–IL-10 blocking Ab was able to restore IFN-α secretion in PBMCs stimulated simultaneously with CpG ODN and LPS (Supplemental Fig. 3). However, in bacterial lysate-stimulated PBMC IFN-α induction was not restored in the presence of an anti–IL-10 blocking Ab (Fig. 5D), suggesting additional factors that contribute to suppression of IFN-α in this setting. Neutralizing Abs for IL-6 and TNF-α, inhibition of IL-1β release by caspase inhibition (z-YVAD), or antagonizing adenosine signaling in PBMCs with caffeine were also not sufficient to restore bacterial lysate-stimulated IFN-α production in PDCs (data not shown). Also, neither heat inactivation nor filtering the supernatants of monocytes substantially reduced their ability to inhibit CpG ODN-induced IFN-α in PDCs (Fig. 5E), indicating that the inhibitory factor was neither a protein nor a membrane vesicle. PGs are heat-resistant small molecule factors that are rapidly released by activated monocytes upon stimulation. Accordingly, we found that hKEC stimulated monocyte supernatant acquired inhibitory activity already within the first 2 h of stimulation (Fig. 5E). We
therefore inhibited cyclooxygenase-dependent PG formation in monocytes using indomethacin and found a significantly reduced ability of the supernatants to inhibit CpG-induced IFN-α production in PDCs (Fig. 5F). Together, these results demonstrate that bacterial lysate-induced TLR9 activation of PDCs is inhibited by factors released from simultaneously activated monocytes and that this inhibition is at least in part due to IL-10 and due to cyclooxygenase-dependent factors such as PG.

Discussion

Purified bacterial DNA induces IFN-α production in PDCs (10, 15), and the detection of unmethylated CpG dinucleotides in certain sequence contexts (CpG motifs) by TLR9 was found to be responsible for this effect (9, 11, 12, 14). However, purified bacterial DNA may induce different immune responses than bacterial DNA in the context of other bacterial TLR ligands. In this paper, we provide evidence that the function of TLR9 in PDCs is actively suppressed in the presence of monocytes simultaneously stimulated by bacterial molecules such as LPS and proteoglycan. The functional consequence is that TLR9-mediated IFN-α induction is not a hallmark of antibacterial but of antiviral responses. This explains why IFN-α is not induced during infection with extracellular bacteria, although lysed extracellular bacteria release abundant bacterial DNA that in principle could stimulate IFN-α in PDCs, were it not for monocyte-derived factors that suppress this TLR9-dependent response.

Our results demonstrate that bacterial lysates induce IFN-α in highly purified PDCs. DNAse treatment of bacterial lysates showed that this effect is largely due to bacterial DNA. Bacterial DNA is detected in the endosome by TLR9; in addition, cytosolic receptors were proposed for the detection of DNA (30). We observed that PDC activation by bacterial lysate was inhibited by CQ, as well as by the TLR9 inhibitory ODN TTAGGG, which indicated that activation was mediated by endosomal TLR9 rather than by cytosolic receptors. However, within PBMCs, monocytes activated by TLR4 (LPS) or TLR2 (proteoglycans) released IL-10-

Table I. Quantitative relation of monocytes and PDCs in human PBMCs

<table>
<thead>
<tr>
<th>Donor</th>
<th>Frequency of Monocytes</th>
<th>Frequency of PDCs</th>
<th>Ratio of Monocytes/PDCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1</td>
<td>15.9</td>
<td>0.69</td>
<td>23</td>
</tr>
<tr>
<td>Donor 2</td>
<td>18.3</td>
<td>0.70</td>
<td>26.1</td>
</tr>
<tr>
<td>Donor 3</td>
<td>12.8</td>
<td>0.58</td>
<td>22.1</td>
</tr>
<tr>
<td>Mean</td>
<td>15.7</td>
<td>0.66</td>
<td>23.7</td>
</tr>
<tr>
<td>SEM</td>
<td>1.59</td>
<td>0.04</td>
<td>1.21</td>
</tr>
</tbody>
</table>

FIGURE 5. PGs and IL-10 contribute to monocyte-mediated suppression of IFN-α induction in PDCs. PBMCs or PDCs were stimulated with CpG ODN 2336 (5 μg/ml) or hkEC (10%, v/v), and IL-10 or IFN-α were measured in the supernatants. A, PBMCs (black bars) or PBMCs depleted of monocytes (gray bars) were stimulated with hkEC or CpG ODN 2336, and IL-10 was measured in the supernatants. B, PBMCs were stimulated with CpG ODN 2336 in the presence or absence of rIL-10 (5 ng/ml). C, PDCs were stimulated with hkEC with or without rIL-10 (2 ng/ml). D, PBMCs were stimulated with CpG ODN 2336 or hkEC in the presence of an anti–IL-10 blocking Ab (αIL-10) or a control Ab (Iso). E, PDCs were stimulated with hkEC in the presence of monocyte-derived supernatants. F, PDCs were stimulated with hkEC in the presence of monocyte-derived supernatants. Data are means ± SEM of three independent experiments (A–C, E, F) or representative of two independent experiments (D). MSN, isolated monocytes stimulated with hkEC; MSN-HI, heat-inactivated supernatants of isolated monocytes stimulated with hkEC; MSN-F, filtered supernatants of isolated monocytes stimulated with hkEC; MSN-2h, supernatants of isolated monocytes stimulated with hkEC for 2 h; MSN-I, isolated monocytes incubated with indomethacin prestimulation with hkEC; MSN-HI/F, heat-inactivated supernatants of isolated monocytes incubated with indomethacin prestimulation with hkEC.

*p < 0.05; **p < 0.01.
and cyclooxygenase-dependent factors like PGs, which blocked responsiveness of PDCs to bacterial DNA or synthetic CpG DNA. Inhibition of PDC function occurred rapidly within the first 2 h of stimulation.

Our results are in agreement with reports by others that IL-10 inhibits the response of PDCs to CpG DNA (31, 32). However, in our hands, IL-10 was not the only factor involved in monocyte-dependent suppression of PDC function, because blocking Abs to IL-10 and heat-inactivation of monocyte-derived supernatants did not restore TLR9 sensitivity of PDCs. Incubation of monocytes with the cyclooxygenase inhibitor indomethacin partially reduced the ability of monocyte-derived supernatants to inhibit PDC function, suggesting that in addition to IL-10, cyclooxygenase-dependent formation of PGs contributes to inhibition of PDCs. Consistent with this finding, tumor-derived PGs were found to inhibit the ability of PDCs to produce IFN-α upon stimulation with CpG DNA (33).

It is interesting to note that a number of viruses were found to express TLR2 ligands (for a detailed review, see Ref. 2). Our findings together with observations by others that IL-10 released upon concomitant TLR2 stimulation blocks the induction of a subset of Th1 cytokines specifically induced by TLR4 or TLR3 in human dendritic cells (34) support the concept that viruses benefit from the expression of TLR2 ligands and thus viral evolution may have actively favored viral proteins that have TLR2 ligand activity to mimic bacterial infection and thus to escape the development of antiviral immune responses. These mechanisms have been demonstrated in detail for hepatitis C virus (35, 36).

PDCs selectively express TLR7 and TLR9, and, within human immune cells, TLR9-induced IFN-α formation is limited to PDCs. Consequently, all of TLR ligands, only TLR7 and TLR9 ligands are capable of inducing IFN-α. The cross-inhibition of PDCs by monocytes secures that the antiviral cytokine IFN-α signaling accompanied by severe impairment of granulocyte function and recruitment. Therefore, TLR9-dependent induction of IFN-α by bacterial DNA during bacterial infections would be harmful to the host, and both restricted expression of TLR9 to PDCs (and B cells) and cross-inhibition of PDCs by concomitantly activated monocytes ensure that IFN-α induction is avoided (despite the presence of TLR9 ligands) and the appropriate antibacterial response is initiated (39). Of note, unlike PDCs, B cells are not inhibited but rather supported by monocyte-derived IL-10. This is well in agreement with the concept that TLR9 is involved in the induction of humoral responses induced by Ag–Ab complexes containing bacterial DNA and other bacterial TLR ligands. In this context, TLR9 is indeed involved in antibacterial immune responses, and the containment of IFN-α production in PDCs seems to play the critical role in avoiding humoral autoimmunity (40). Furthermore, it should be noted that in mice, TLR9 is not limited to PDCs and B cells, but is also expressed in myeloid immune cells, and that this different expression pattern may explain differences seen for bacterial lysates between mouse and man (41).

A broadly accepted paradigm is that bacterial DNA is the active ingredient in bacterial lysates used for immunotherapy of tumors (16). In 1893, Sir William Coley reported successful treatment of tumors by the injection of bacterial lysates (7). Tokunaga and colleagues (38) demonstrated that viral infection predisposes for bacterial superinfections and that such increased susceptibility to bacterial infections is due to IFN-α signaling accompanied by severe impairment of granulocyte function and recruitment. Therefore, TLR9-dependent induction of IFN-α by bacterial DNA during bacterial infections would be harmful to the host, and both restricted expression of TLR9 to PDCs (and B cells) and cross-inhibition of PDCs by concomitantly activated monocytes ensure that IFN-α induction is avoided (despite the presence of TLR9 ligands) and the appropriate antibacterial response is initiated (39). Of note, unlike PDCs, B cells are not inhibited but rather supported by monocyte-derived IL-10. This is well in agreement with the concept that TLR9 is involved in the induction of humoral responses induced by Ag–Ab complexes containing bacterial DNA and other bacterial TLR ligands. In this context, TLR9 is indeed involved in antibacterial immune responses, and the containment of IFN-α production in PDCs seems to play the critical role in avoiding humoral autoimmunity (40). Furthermore, it should be noted that in mice, TLR9 is not limited to PDCs and B cells, but is also expressed in myeloid immune cells, and that this different expression pattern may explain differences seen for bacterial lysates between mouse and man (41).

ACKNOWLEDGMENTS

We thank B. Lüdenbach, S. Riemann, and M. Stasch for expert technical support with the experiments.

DISCLOSURES

The authors have no financial conflicts of interest.

REFERENCES