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Decreased Pathology and Prolonged Survival of Human DC-SIGN Transgenic Mice during Mycobacterial Infection

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Dendritic cell (DC)-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN: CD209) is a C-type lectin that binds ICAM-2,3 and various pathogens such as HIV, helicobacter, and mycobacteria. It has been suggested that Mycobacterium tuberculosis, the causative agent of pulmonary tuberculosis, interacts with DC-SIGN to evade the immune system. To directly analyze the role of human DC-SIGN during mycobacterial infection, we generated conventional transgenic (tg) mice (termed “hSIGN”) using CD209 cDNA under the control of the murine CD11c promoter. Upon mycobacterial infection, DCs from hSIGN mice produced significantly less IL-12p40 and no significant differences were observed in the secretion levels of IL-10 relative to control DCs. After high dose aerosol infection with the strain M. tuberculosis H37Rv, hSIGN mice showed massive accumulation of DC-SIGN+ cells in infected lungs, reduced tissue damage and prolonged survival. Based on our in vivo data, we propose that instead of favoring the immune evasion of mycobacteria, human DC-SIGN may have evolved as a pathogen receptor promoting protection by limiting tuberculosis-induced pathology. The Journal of Immunology, 2008, 180: 6836–6845.

World-wide, it is estimated that every second, another human being is infected with Mycobacterium tuberculosis (Mtbb) and with one third of the world’s population latently infected, Mtbb is considered one of the most successful pathogens (1, 2). IL-12 producing dendritic cells (DCs) are critical for immune responses and play an essential role in mounting cellular immune responses against mycobacteria (4–10). DCs recognize mycobacteria through a variety of germline-encoded pattern recognition receptors including TLRs (reviewed in Ref. 11) and lectins such as DC-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN: CD209) (12, 13).

TLR2, TLR4, and TLR9 have been shown to be critically involved in mycobacteria-induced cell activation (14–17). TLR2 mediates cellular activation after stimulation with mycobacterial lipoproteins and terminally nonmannosylated lipoarabinomannan (LAM) (18, 19), whereas TLR4 is involved in signaling events induced by a heat-sensitive cell-wall associated mycobacterial factor (16). If the mycobacterial DNA, is essential for mounting a Th1 response against Mtbb and cooperates with TLR2 in vivo.

DC-SIGN, a C-type lectin, is a type II transmembrane protein mainly expressed on myeloid DCs and contains a carbohydrate recognition domain important for pathogen binding (20). In primates, the intracellular domain is characterized by the presence of a highly conserved di-leucine/tri-acidic cluster internalization signal and an incomplete tyrosine-based ITAM (21). Several mouse homologues have been cloned (22–24) which all lack the putative internalization and signaling motifs that in primates were under strong selective constraints preventing amino acid changes during evolution (25). Currently, however, the cellular expression pattern has only been elucidated for two of those homologues: CD209a (mDC-SIGN) has an unknown function on plasmacytoid pre-DCs and macrophages (26), whereas CD209b (SIGN-R1) is present on macrophage subpopulations in spleen and lymph nodes, but not in the lung and has been shown to be unimportant in mouse models of Mtbb infection (27, 28).

Binding of mycobacterial mannosylated LAM (ManLAM) to human DC-SIGN induces a block on LPS-induced maturation of human DCs and leads to enhanced release of the anti-inflammatory cytokine IL-10 (12). Based on these findings, it was proposed that...
mycobacterial cell wall components are able to modulate TLR-signaling by a DC-SIGN-dependent mechanism which may con-tribute to the immune evasion of Mtb (21). Genetic epidemiology has yielded conflicting data. A recent study by Vannberg et al. (29) suggested that human single nucleotide polymorphisms (SNPs) leading to decreased DC-SIGN expression are associated with re-duced risk for cavitary tuberculosis disease, while a previous pub-lication demonstrated that SNPs in the CD209 promoter region leading to increased DC-SIGN expression were protective against tuberculosis (30).

Therefore, to address the functional role of human DC-SIGN under experimental in vivo conditions, we generated mice trans-genic for human DC-SIGN (termed “hSIGN”), in which DC-SIGN is expressed under the control of the CD11c promoter, and ana-lyzed these mice in mycobacterial infection models. Interestingly, in contrast to data obtained with human cells, in vitro myco-bacterial infection DCs from hSIGN mice did not secrete higher amounts of IL-10, and produced significantly less IL-12. In addi-tion, during chronic Mtb infection in vivo, hSIGN mice reduced skin tissue damage and survived significantly longer. We con-clude therefore that human DC-SIGN may be an evolutionary con-served molecule in primates, because instead of favoring immune evasion of mycobacteria, it appears to limit the elicited pathology during chronic tuberculosis infection.

Materials and Methods

Constructs

Human DC-SIGN transgenic (tg) mice were generated using a human DC-SIGN cDNA sequence driven by the murine CD11c promoter that was kindly provided by Dr. Thomas Brocker (Ludwig-Maximilians University, Munich, Germany) (31). A 1.3-kb cDNA fragment of human DC-SIGN (CD209) was amplified by PCR using total cDNA from human peripheral blood monocyte-derived dendritic cells as template. The cDNA was cloned into the 3’ of the 5.5-kb CD11c promoter via EcoRI restriction sites. The construct was linearized by a combined NotI/Clal digest and purified by gel elec-trophoresis for microinjection into fertilized 6D6Z/F1 hybrid oocytes.

Mice

C57BL/6 mice were purchased from Harlan Winkelmann. Human DC-SIGN tg founder mice (termed “hSIGN”) were identified by PCR using primers 5’-AGG TGG GAT CTG CGg AAg ggg gTG ACA TAg gT gACT-3’ and 360: 5’-ACg CGg TAG gAg gAA AAg ggc gAA TgT gTg TAC g-3’, selection was based on the levels of transgene expression in DCs, and backcrossed for 10 generations on C57BL/6 background. Animals were bred and maintained under SPF conditions at the animal facility of the Institute for Medical Microbiology, Immunology, and Hygiene, Technical University of Munich. Sex- and age-matched tg and littermate control mice between 6- and 14-wk of age were used in all experiments. Mtb infection experiments were performed in the biosafety level III facilities at the Di-vision of Molecular Infection Biology, Research Center Borstel. All animal experiments were performed under specific pathogen-free conditions and in accordance with institutional, state, and federal guidelines.

Cells

Bone marrow Flt3-ligand- and GM-CSF-derived (GM-DC) bone marrow dendritic cells (BMDCs) were generated as described previously (32, 33). In brief, bone marrow cells were removed from femurs and tibias of mice and cultured in complete RPMI (RPMI 1640, 2 mM 1-glutamine, heat-inactivated 10% FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME (all from PAA Laboratories GmbH). All cells were costained with propidium iodide or ethidium monoazide to exclude nonviable cells (Sigma-Aldrich). Cytometric analysis was performed using a FACSCalibur flow cytometer (BD Biocytom) and CellQuestPro (BD Biosciences). Subsequent data analysis was performed with FlowJo software (Tree Star).

ELISPOT assay for IFN-γ

For Ag-specific restimulation, single-cell suspensions of lungs were prepared from Mtb-infected mice at different time points. Mice were anesthe-tized with a lethal dose and lungs were perfused through the right ventricle with warm PBS. Once lungs appeared white, they were removed and sec-tioned. Dissected lung tissue was incubated in 0.7 mg/ml collagenase A (Roche Diagnostics GmbH) and 30 μg/ml DNase (Sigma-Aldrich) at 37°C for 2 h. Digested lung tissue was gently disrupted by subsequent passage through a 100-μm pore size nylon cell strainer. Recovered lung cells were depleted of remaining erythrocytes and resuspended in complete IMDM for additional experiments. CD4 or CD8 T cells of seven infected mice per genotype where pooled into three to four groups. From single cell suspensions, CD4 or CD8 T cells were enriched by magnetic cell sorting (Miltenyi Biotec) and IFN-γ production was detected by an ELISPOT assay kit (AID). In brief, enriched CD4 or CD8 T cells were seeded at an initial concentration of 1 × 10^5 cells/well into anti IFN-γ mAb-coated plates and serially diluted in complete IMDM. Total splenocytes from uninfected WT mice using magnetic beads (CD4 untouched-kit, Invitrogen) and 2 × 10^6 CD4 T cells were added to the wells containing Mtb3293–102 were added to the wells containing CD8 T cells. After 24 h incubation in 5% CO₂ at 37°C, plates were washed and bound cytokine was detected using a biotinylated anti-IFN-γ mAb (Mabtech). Spots were visualized using streptavidin-HRP and AEC (AID) as substrate. Frequency of re-sponding cells was determined by using an AID ELISPOT Reader System ELR02 (AID). By plotting the frequency of spot forming cells against the Beijing strains (3547/01;4203/01) were grown at 37°C in Middlebrook 7H9 broth supplemented with Middlebrook OADC-Enrichment (BD Biosciences). Midlog phase cultures were harvested, aliquoted, and frozen at −80°C. Viable cell counts were determined by plating serial dilutions of cultures on Middlebrook 7H10 agar plates (BD Biosciences). For experi-ment in vitro infections, aliquots were diluted in culture media and the preparation was passed six times through a 27-gauge needle to ensure proper dispersion of mycobacteria. Day 9 BMDCs were stimulated for 24 h with M. bovis BCG or Mtb at different multiplicities of infection. For DC-SIGN stimulation, 3 × 10^5 CD4 T cells and 7 DCs from hSIGN and wild type (WT) mice were harvested, washed, and infected with 50 and 10 multiplicities of infection of M. bovis BCG. After overnight culture, DCs were washed, resuspended in complete RPMI 1640, and plated on 96-well plates at a density of 1 × 10^5 DCs/well. CD4 T cells were purified from lymphnodes of C57BL/J WT mice using magnetic beads (CD4 untouched-kit, Invitrogen) and 2 × 10^6 CD4 T cells were added to 1 × 10^5 DCs/well. After 24 h, supernatants were taken and frozen at −80°C until analysis.

Pulmonary infection was performed using an inhalation exposure sys-tem (Glas-Col). Mice were infected with a dose of 100, 1000, or 2000 CFU/lung, confirmed by determining the bacterial load in undiluted homogenates of the entire lung 24 h after infection. Mice were regularly weighed and monitored for survival and clinical signs. Before and after infection, blood was collected by tail puncture. Dissected lung tissue was incubated in 0.7 mg/ml collagenase A (Roche Diagnostics GmbH) for 1 h at 37°C. Viable cell counts were determined by determining the bacterial load in undiluted homogenates of lungs. 50 μg/ml Pam3CSK4 (EMC Microcollections GmbH).

Flow cytometry

To block Fc-receptors, cells were incubated with unlabeled anti-CD16/ CD32 (93, eBioscience, Frankfurt, Germany) Ab for 10 min at 4°C. Staining was performed with Abs against murine CD11c (N418, eBioscience), murine CD45R (RA3– 6B2, eBioscience), murine MHC class II (MHCII; MS114.15.2, eBioscience, Frankfurt, Germany) and human CD209 (120507, R&D Systems GmbH). All cells were costained with propidium iodide or ethidium monoazide to exclude nonviable cells (Sigma-Aldrich). Cytometric analysis was performed using a FACSCalibur flow cytometer (BD Biosciences) and CellQuestPro (BD Biosciences). Subsequent data analysis was performed with FlowJo software (Tree Star).
total number of T cells seeded into the well, a linear regression was performed and the number of spot forming cells per 100000 cells was calculated.

**Cytokines and metalloproteinases**

Culture supernatants were harvested 24 h after infection with mycobacteria and frozen at −80°C until analysis. IL-10, IL-12p40, IFN-γ, and TNF-α levels were determined by ELISA (Duo Set, R&D Systems). RNA was isolated from lung homogenates of infected mice at the indicated time points, reverse transcribed, and analyzed using quantitative Lightcycler Technology. We monitored matrix metalloproteinase (MMP) activity using a fluorogenic peptide as substrate for MMPs according to the manufacturer’s instructions.

**Lang histochemistry**

Lungs were fixed ex situ with 4% (w/v) phosphate buffered paraformaldehyde via the trachea, removed, and stored in 4% paraformaldehyde. To obtain a representative collection of lung tissue samples, systematic uniform random (SUR) sampling was performed. SUR samples were embedded into paraffin, and 2-μm sections were stained for anti-human DC-SIGN (BD Biosciences) by indirect immunohistochemistry. Standard H&E or Elastica-van-Gieson staining was performed for quantitative morphological analysis of histopathological alterations. One section of each SUR sample was analyzed using a computer-assisted stereology tool box (Visiopharm), and the following parameters were recorded from meander sampled SUR fields of view according to established stereological methods (37); arithmetic mean thickness of airway epithelium, volume of intrabronchial debris per epithelial membrane area, and surface fraction of bronchial elastic membrane gaps relative to total epithelial membrane area. To identify the phenotype of DC-SIGN+ cells, four consecutive sections of each block were stained as follows: section 1 by Ziehl-Neelsen staining (TB-Kinyoun staining kit, BD Biosciences), and sections 2 and 3 by indirect immunohistochemistry for anti-human DC-SIGN (EB-h209, ebioscience) and anti-mouse MHCII (2G9, BD Biosciences), respectively. As murine alveolar epithelial type II cells are also known to express MHCII upon mycobacterial infection (38), section 4 was stained by indirect immunohistochemistry for anti-human surfactant protein B (Chemicon). In addition, double stainings for anti-human DC-SIGN followed by Ziehl-Neelsen staining were performed to reveal whether DC-SIGN+ cells were infected with mycobacteria or not.

**Results**

**Generation of hSIGN tg mice**

In humans, DC-SIGN is mainly expressed by myeloid DCs in mucosal tissues and can be detected on human blood-derived DCs after in vitro differentiation with IL-4 and GM-CSF as well as on CD11c+ myeloid alveolar lavage cells of Mtb infected patients (21, 39). To drive tissue-specific expression of the human DC-SIGN transgene in murine DCs, we used the CD11c minimal promoter (31) for generating conventional tg mice and introduced the cDNA coding for CD209 via EcoRI restriction sites (Fig. 1A). The construct was injected into the pronuclei of fertilized B6D2F1 hybrid oocytes. Tg mouse lines with high transgene expression were established and termed hSIGN mice. Flow cytometric analysis of GM-CSF- and Flt3-ligand BMDCs from hSIGN mice revealed specific expression within MHCII+CD11c+ DCs, with highest expression within conventional CD11c+CD45R+ DCs and only weak expression in CD11c+CD45R− plasmacytoid pre-DCs (Fig. 1B).

Flow cytometric analysis of DC-enriched spleen and LN cells also demonstrated that the human transgene is faithfully expressed in all CD11c+CD45R− DCs and is excluded from CD11c− cells (Fig. 1C, and not depicted). High transgene expression could be observed in all CD4+ and CD8+ T cells, and CD4+ CD8+ conventional DC subsets (not depicted). Expression level in ex vivo isolated splenic CD11c+CD45R+ plasmacytoid pre-DCs was low (Fig. 1C), which closely resembles the expression pattern of DC-SIGN in conventional human DCs.

For localization of human DC-SIGN tg cells in situ, tissue sections of lymph node, spleen, and lung were analyzed by immunohistochemistry. Cells positive for human DC-SIGN were present in the T cell areas of spleens and lymph nodes of hSIGN mice and showed characteristic DC morphology. Human DC-SIGN expression was not detectable in the germinal centers and marginal zones of the lymphoid tissues. Lung sections of naive hSIGN mice revealed that cells positive for human DC-SIGN were located in the adventitial layer of the airway walls, focally present in alveolar septal walls, and rarely seen as intraepithelial cells of the main bronchi (Fig. 2). Taken together, hSIGN mice show major expression of the human transgene in conventional DCs, which are rarely found in the lungs of uninfected mice.

**FIGURE 1.** Generation and characterization of human DC-SIGN tg mice. A. Schematic structure of the human DC-SIGN tg construct. Human DC-SIGN cDNA was placed under the control of the murine CD11c promoter via EcoRI restriction sites. B. CD209 expression on in vitro generated BMDCs. GM-CSF-BMDCs were analyzed for surface expression of CD11c/MHCII and CD209, whereas Flt3-ligand BMDC were stained for CD11c/CD45R/C2D09. Histograms are gated on CD11c+MHCII and CD209, whereas Flt3-ligand BMDCs were stained for CD11c/CD45R/C2D09. Open histograms, CD209; gray filled histograms, isotype control. C. CD209 expression on ex vivo enriched splenic cells. DCs were isolated from spleen of wt or hSIGN mice. Nycodenz (67) enriched DCs were stained for CD11c, CD45R, and CD209 markers and analyzed for CD209 expression in the indicated populations. Open histograms, CD209; grey tinted histograms, isotype control. Data represent one experiment from three.
Reduced IL-12 secretion by hSIGN \(^+\) DCs after mycobacterial infection in vitro

Because triggering of DC-SIGN has been shown to affect TLR4-mediated cellular responses of human DCs leading to increased IL-10 production (12), we infected GM-CSF-derived BMDCs from WT and hSIGN mice with different doses of \(M.\) bovis BCG and measured subsequent IL-10 levels after 24 h (Fig. 3A). TLR-stimuli alone (CpG, LPS, and Pam \(_{3}\)CSK\(_{4}\)) served as controls. No differences in IL-10 production could be observed between WT and tg DCs at 24 h after BCG infection or earlier time points (Fig. 3A and not depicted). Using the same stimulus, no gross differences were observed with regard to the surface expression of co-stimulatory molecules (CD80, CD86) and DC maturation markers (MHCII) (not depicted). Also, cell wall components or ManLAM from \(Mtb\) H37Rv in combination with titrated amounts of LPS did not trigger increased IL-10 secretion from \(tg\) DCs (not depicted).

Because IL-12 plays a pivotal role in the control of mycobacterial infection (40, 41), we also measured IL-12p40 using the same experimental setup. In this study, in comparison to the IL-12p40 formation of DCs from WT mice in response to \(M.\) bovis BCG, the IL-12p40 released from hSIGN \(tg\) DCs was significantly reduced by 50–70% (Fig. 3A). Stimulation of BMDCs with cell wall components from \(Mtb\) H37Rv also resulted in reduction of IL-12 in hSIGN BMDCs (not depicted), suggesting that infection of DCs with live bacteria is not required and that the surface expression of the human transgene changes the cytokine profile after DC-SIGN and TLR cross-linking. Using defined TLR-agonists for TLR2 (Pam \(_{3}\)CSK\(_{4}\)), TLR4 (LPS), or TLR9 (CpG) as DC-SIGN-independent DC stimuli, we observed equivalent cytokine responses in WT and DC-SIGN \(tg\) BMDCs confirming that TLR signaling is not influenced if DC-SIGN ligation does not occur.
similar experiments with different strains of using pathologically relevant mycobacterial strains, we performed (Table I). M. bovis DCs (5–40 min) could be detected (not depicted). In summary, /H9253 and IFN- secretion by T cells primed with hDC-SIGN Mtb bacteria (data not shown). Furthermore, no differences in I activation during acute BCG infection of /H11006 A. CFU amounts were determined in the lungs at indicated time points after infection with 100 CFU Mtb. B. Mycobacterial load in the lung, liver, and spleen was determined on day 21 and 42 days after aerosol infection with 2000 CFU Mtb.

To test whether reduction of IL-12p40 levels was also observed using pathologically relevant mycobacterial strains, we performed similar experiments with different strains of Mtb including the widely used lab strain Mtb H37Rv as well as Mtb CDC 1551, which was isolated during an outbreak of TB close to the Kentucky-Tennessee border (42). In addition, two clinical Mtb isolates of the Beijing genotype (43), from TB patients from Afghanistan (3547/01) and Vietnam (4203/01), were included in this study. Supernatants of infected DCs were analyzed for IL-12p40 and IFN- secretion by T cells primed with hDC-SIGN tg DCs compared with WT cells (data not shown). Importantly, murine bone marrow-derived macrophages from hSIGN mice lacking hDC-SIGN expression displayed similar cytokine profiles to macrophages from WT mice (data not shown). Next, we wanted to analyze whether reduced IL12 production by tg DCs may affect the priming of naive T cells during DC-T cell cocultures. We infected DCs from WT and hSIGN mice with different doses of M. bovis BCG and added naive CD4 T cells to the cultures 14 h after infection. Supernatants of cocultures collected at 38 h after infection demonstrated decreased IL12p40 levels from infected DCs as well as decreased IFN- secretion by T cells primed with hDC-SIGN tg DCs compared with T cells cocultured with WT DCs (Table I).

To address the question of whether TLR signaling pathways involved in IL-12 production were affected by the human transgene, we examined the influence of human DC-SIGN on the activation of MAPK known to be critically involved in the release of mycobacteria (data not shown). Furthermore, no differences in IκB degradation and NFκB activation during acute BCG infection of DCs (5–40 min) could be detected (not depicted). In summary, while putative DC-SIGN-mediated signaling pathways await further elucidation, in DCs from hSIGN mice the main cytokine driving Th1 cellular immune responses is significantly reduced after stimulation with both BCG and Mtb. This humanized mouse model now allowed us to study the in vivo relevance of DC-SIGN during mycobacterial infection.

No differences in mycobacterial load between WT and hSIGN infected mice

To investigate the course of Mtb infection in the humanized mouse model, the mycobacterial load was analyzed after aerosol infection of WT and hSIGN mice with 100 CFU Mtb H37Rv at different time points (Fig. 4A). In both mouse strains, the replication rate of mycobacteria between day 1 and day 21 was of a similar magnitude. At day 21, the CFU level reached a plateau of $1 \times 10^7$ bacteria, which remained almost unchanged until day 90. At all time points, no differences could be detected in the bacterial burden of lungs prepared from WT and human DC-SIGN tg mice (Fig. 4A). In a previous study, it was demonstrated that TLR2-deficient mice had a reduced resistance to Mtb infection only with a very high inoculum (46). Therefore, mice were infected per aerosol with 2000 CFU Mtb (Fig. 4B). After 21 days, the bacterial burden reached $1 \times 10^5$ bacteria per lung. However, both groups of infected mice possessed equal bacterial loads in the lungs, spleens, and livers on day 21 and 42 (Fig. 4B). Similar results were obtained on day 14 p.i. when WT and hSIGN mice were infected i.p. with $1 \times 10^7$ M. bovis BCG (not depicted). Hence, despite
differences in IL-12 secretion from in vitro cultured DCs, we did not detect differences in the mycobacterial load between WT and hSIGN mice after aerosol infection. To monitor cytokines directly ex vivo, we also measured lung homogenate mRNA and protein expression of various mediators, which are known to be important for mounting resistance after Mtb infection. However, no gross differences in expression of key effector molecules such as TNF-α, IL-10, IFN-γ, inducible NO synthase, and MMP 2 were observed in whole lung homogenates (data not shown). In contrast to the results obtained after Mtb infection of DCs in vitro, there was also no difference in the levels of IL-12p40 expression in vivo, attesting to a lack of sensitivity of whole lung homogenates or to the enhanced plasticity of immune responses and other possible cellular sources of IL12-p40 in vivo (not depicted).

Delayed priming of Mtb-specific CD8⁺ T cells

To analyze whether the priming of T cells would differ between WT and hSIGN mice in vivo, we incubated APCs with purified CD4⁺ and CD8⁺ cells from lymph nodes or lungs of Mtb-infected mice in the presence of Mtb-derived peptides (ESAT6, Mtb32) and measured IFN-γ production by ELISPOT. The total number of CD4⁺ IFN-γ-producing cells in either lymph node or lung did not differ between WT and hSIGN mice, in line with our finding that the total amount of IFN-γ mRNA and protein in Mtb-infected mouse lungs was similar in WT and hSIGN mice. In contrast, the number of Mtb-specific, CD8⁺ IFN-γ-producing cells was significantly reduced in the lungs of hSIGN mice, particularly on day 21 after infection (Fig. 5). A similar trend was seen in CD8⁺ cells isolated from lymph nodes, however this difference did not reach statistical significance (data not shown). Because the total number of CD3/CD28-stimulated IFN-γ-producing CD8⁺ cells was similar in WT and tg mice (data not shown), these data suggest that the priming of Mtb-specific CD8 cells is impaired or delayed in hSIGN mice.
day 148 the lungs of WT mice showed massive damage to the lung parenchyma and distal airways as indicated by a marked increase in necrotic tissue and cell debris occluding distal and mid-level bronchi (Fig. 7, G and H). In addition, Elastica-van-Gieson staining revealed an increase in elastic membrane gaps of airways after 148 days of infection in WT mice but not hSIGN mice (Fig. 7, I and J). Stereological analysis of histological sections, which were collected by systematic uniform random sampling to assure that the fields of view analyzed were representative of the whole organ, revealed a marked increase in elastic membrane breaks \( p = 0.01 \), a significantly enhanced accumulation of cellular debris within bronchi and bronchioli \( p < 0.001 \), and a decrease in airway epithelium thickness \( p < 0.02 \) between day 42 and 148 after Mtb infection in WT but not hSIGN mice (Fig. 8). Critical mediators of tissue reorganization and, if dysregulated, destruction are MMPs (reviewed in Ref. 47). We therefore monitored MMP activity using a fluorogenic peptide as substrate for MMP-1, MMP-2, MMP-7, MMP-8, MMP-9, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, and Cathepsin D and E. Although a time dependent increase in MMP activity in lung homogenates after Mtb infection was observed, there was no difference between WT and DC-SIGN tg mice after day 148 (data not shown). Analysis of the bacterial burden in the lungs of these mice did not reveal a significant difference between WT and DC-SIGN tg mice (WT: 7.707 ± 0.274 CFU (log10/lung), DC-SIGN tg 7.182 ± 0.275 CFU (log 10/lung); Mean ± SD, \( p = 0.0571 \); Mann-Whitney U Test).

**Prolonged survival of hSIGN mice during chronic Mtb infection**

The observations that the presence of human DC-SIGN: 1) limits the production of the proinflammatory cytokine IL-12 by BMDCs after infection with Mtb in vitro and 2) has a beneficial effect on the pathology elicited by Mtb infection, raised the question whether DC-SIGN would have an impact on long term survival of Mtb infected mice tg for human DC-SIGN. Until approximately day
and hSIGN mice were aerogenically infected with 100 (A) and 2000 (B) CFU *Mtb*. Ten mice per group were monitored and moribund mice were sacrificed. Statistical analysis of the survival curve was performed using log-rank test. *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001; n.s., not significant.

330 after infection with 100 CFU *Mtb*, both WT and hSIGN mice demonstrated a similar survival rate (Fig. 9A). Thereafter, whereas 50% of infected hSIGN animals survived until day 390, 50% of all WT animals had died by day 370. After day 390, mice bearing hDC-SIGN died at a lower rate than WT mice. All WT mice were dead by day 440, while 40% of hSIGN mice were still alive at this time point. However, these differences were not statistically significant. In a second experimental setting, both mouse strains were infected with a higher dose of 2000 CFU *Mtb*. Until day 100, no differences in the survival rate of infected C57BL/6 and hSIGN mice was observed (Fig. 9B). At day 150 almost 50% of the infected WT mice had succumbed to the infection. In comparison, hSIGN mice demonstrated a prolonged 50% survival rate of ~200 days. Whereas all WT mice had died by day 190 after infection, the last hSIGN mouse died on day 270. Therefore, in this high-dose challenge model, a high propensity of survival was observed in mice bearing the human DC-SIGN (log rank test; *p* < 0.001).

**Discussion**

Animal models of low-dose aerosol infection with *Mtb* are believed to reflect the typical infection that occurs when humans inhale only a few virulent bacteria aerosolized in the course of an infected individual’s coughing (48, 49). However, the use of mice as model organisms for the study of immune responses during infectious diseases carries the risk that important host-pathogen interactions may be overlooked or misinterpreted, for example if the corresponding pathogen receptor is not expressed in mice. Humanized mice, which mimic human physiological systems after genetic manipulation, are therefore becoming important tools in the analysis of infectious human diseases (50, 51).

Based on in vitro studies using human DCs it has been hypothesized that in mycobacterial infection human DC-SIGN mediates immune evasion by inhibiting TLR-signaling (12). Whereas TLR-signaling is highly conserved between mice and men, none of the DC-SIGN mouse homologues are expressed on GM-CSF bone marrow-derived myeloid DCs in mice (our unpublished data). In addition, all mouse homologues lack the putative signaling and internalization motifs that are highly conserved in primates (21). To resolve these conflicting issues and gain further insight into a possible role of human DC-SIGN after infection with *Mtb* in vivo, we have generated a hSIGN tg mouse model. Immunohistochemical stainings of lung sections from naive hSIGN mice showed expression of human DC-SIGN on cells of DC-like morphology demonstrating the expression of hDC-SIGN on murine DCs. In addition, hDC-SIGN cell surface expression could be identified directly on ex vivo isolated cells and on DCs from in vitro-differentiated GM-CSF or Flt3L bone marrow cells from hSIGN mice. Taken together, the expression of hDC-SIGN on murine DCs demonstrates that CD11c-driven expression of human DC-SIGN is an appropriate model system to characterize the functional role of hDC-SIGN in murine DCs in vitro and in vivo.

The priming of a Th response into Th1 or Th2 differentiation by DCs is mediated via the expression of different costimulatory molecules on the cell surface and the release of cytokines (52). Particularly the production of IL-12 has been shown to be important for DCs to drive a Th1 response by CD4+ T cells (53). Several independent studies have demonstrated the importance of IL-12 for the induction of an effective immune response after infection with *Mtb*. The initial control of an infection is correlated with the presence of IFN-γ-producing CD4+ T cells (54), which is induced by IL-12 released from infected APCs (40). As shown by Cooper and colleagues, the inability of IL-12p40-deficient mice to control mycobacterial infection is linked to a deficiency in IFN-γ-producing T cells. Another soluble factor secreted during infection with *Mtb* is IL-10 (55). Adoptively transferred IL-10-deficient DCs infected with *M. bovis* BCG show an improved migration to the draining lymph node in WT animals and enhanced induction of IFN-γ-producing T cells in response to mycobacterial Ag, indicating that during mycobacterial infection, immune responses by DCs can be suppressed by autocrine IL-10 via limiting DC migration and IL-12 production (56). In the current study, we observed a significantly reduced level of IL-12p40 production by myeloid BMDC from human DC-SIGN tg animals compared with WT mice after infection with *M. bovis* BCG or different strains of *Mtb*. In contrast to the in vitro data using human dendritic cells (12), a significant human DC-SIGN dependent increase in IL-10 production was not detectable after mycobacterial stimulation, which may be due to intrinsic dissimilarities between murine and human DCs. Differences to the previously reported results could also be a consequence of the lack of interaction of human DC-SIGN with murine adaptor or signaling molecules. First preliminary data suggest, however, that in transgenic DCs, human DC-SIGN triggers similar signaling pathways as detected in human DCs after DC-SIGN ligatation. Those studies are currently under investigation and will shed light on the mechanism of action of human DC-SIGN. The fact that *Mtb*-infected hSIGN mice do not differ from WT mice with regard to IL-12p40 expression levels in the lung suggests that in vivo during chronic infection hDC-SIGN independent compensatory mechanisms inducing IL-12 and hDC-SIGN+ producers of IL-12 may be present. It is equally possible that even though the overall IL-12p40 levels do not differ between infected WT and hSIGN mice, regional differences do exist, for example at the granuloma fringes where newly recruited hDC-SIGN bearing cells are found. These more subtle and compartmentalized variations of cytokine levels may have escaped detection by mRNA or protein measurements in total lung homogenates, but may still have profound consequences for the regulation of DC-dependent immune responses. Interestingly, however, using IFN-γ production as a read-out for Ag-specific effector function, we found CD8 cells.
from hSIGN mice to be significantly less active than CD8 cells from WT mice, possibly suggesting that DC-SIGN may be a hitherto underappreciated factor for the priming of CD8 T cells in tuberculosis. Although IFN-\(\gamma\) secretion by CD8 cells certainly contributes to macrophage activation and tissue damage, lysis of incompetent infected target cells by Ag-specific CD8 cells may also be beneficial, because the released bacteria are then taken up by activated macrophages within the granuloma, and presumably contained or destroyed (57, 58). The role of CD8 T cells in \(Mtb\) infection is most often viewed as a protective one especially during the late phase of experimental infection (59–63), however cytotoxic cells have also been implicated in the pathogenesis of disease because of their potential to cause tissue damage (64), possibly explaining the reduced tissue damage in hSIGN mice. We did not directly measure levels of CD8-mediated cytotoxicity in the lungs of \(Mtb\)-infected hSIGN mice, but instead used \(Mtb\)-specific IFN-\(\gamma\) secretion as a surrogate marker for CD8 T cell activity. Because hSIGN mice exhibited significantly less structural damage to the lung during \(Mtb\) infection than WT mice, it is tempting to speculate that this may have been caused by the recorded dampened CD8 response. To what extent this modulated CD8 response is also causally linked to the longer survival of hSIGN mice needs further detailed examination of cytotoxic effector functions of CD8 cells late during pulmonary \(Mtb\) infection.

The role of IL-12 in murine mycobacterial infections is probably more complex than previously thought: On the one hand it has been demonstrated that a complete loss of the IL-12p40 gene leads to a dramatic reduction in the development of IFN-\(\gamma\) producing T cells during mycobacterial infection. As a consequence, these animals cannot control the mycobacterial growth and die early after infection (40). The opposite situation is characterized by a significant increase in IL-12-as observed in WSX 1-deficient mice after infection with \(Mtb\) (65) or in TNFR1-deficient mice infected with \(Mycobacterium avium\) (66) which provoked accelerated death due to hyperinflammatory responses. Therefore, neither the complete loss of IL-12 nor an excessive inflammation induced by uncontrolled production of this cytokine is of advantage in fighting mycobacterial infection. Combining the presented in vitro and in vivo data, we conclude that the presence of human DC-SIGN is of benefit for the murine host, probably by regulating the inflammatory response after infection. The observation that after infection with \(Mtb\) human DC-SIGN-tg mice survived longer and demonstrated decreased epithelial damage associated with less severe occlusion of the airways in the lungs indicates a role for DC-SIGN in preventing \(Mtb\)-induced pathology and tissue destruction. This interpretation is in line with genetic data published by Barreiro et al. (30) which underscore the importance of DC-SIGN in human infection with \(M. tuberculosis\): The promoter variants (−871G and −336A) of CD209 are associated with a higher expression rate of DC-SIGN are protective during tuberculosis infection arguing against a role for human DC-SIGN in immune evasion of mycoactria (30).

Together, the data presented in this study indicate that human DC-SIGN plays a significant role in balancing the immune response after infection with \(Mtb\). Although strong inflammatory reaction stimulates a faster clearance of the pathogen it simultaneously leads to severe tissue damage and subsequent death. These responses appear to be dampened by expression of human DC-SIGN. This reduced, but not abolished, proinflammatory response would allow the pathogen to survive and reach a chronic but controlled state. The advantage would be the formation of less severe organ damage enabling the host to develop a stronger resistance toward mycobacterial infection.

The human DC-SIGN tg model presented in this study demonstrated a reduced IL-12 production by DCs infected with \(Mtb\) in vitro and a prolonged survival accompanied by less destructive lesion formation in the lungs of infected mice in vivo. The current study did not show a difference in mycobacterial loads between hSIGN and WT mice, which may indicate that DC-SIGN does not primarily act as a vehicle for \(Mtb\) to evade the immune system. Instead, our data suggest that human DC-SIGN may have evolved as a receptor, which enables the host to survive longer by limiting the tissue-damaging inflammatory response. The precise mechanism by which DC-SIGN modulates excessive inflammation may provide important clues for developing strategies to fight tuberculosis and to improve vaccinations.

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Disclosures

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