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Fucose-based PAMPs prime dendritic cells for follicular T helper cell polarization via DC-SIGN-dependent IL-27 production

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Dendritic cells (DCs) orchestrate antibody-mediated responses to combat extracellular pathogens including parasites by initiating T helper cell differentiation. Here we demonstrate that carbohydrate-specific signalling by DC-SIGN drives follicular T helper cell (T_{FH}) differentiation via IL-27 expression. Fucose, but not mannose, engagement of DC-SIGN results in activation of IKKε, which collaborates with type I IFNR signalling to induce formation and activation of transcription factor ISGF3. Notably, ISGF3 induces expression of IL-27 subunit p28, and subsequent IL-27 secreted by DC-SIGN-primed DCs is pivotal for the induction of Bcl-6$^+$CXCR5$^+$PD-1^{hi}Foxp1^{lo} T_{FH} cells, IL-21 secretion by T_{FH} cells and T-cell-dependent IgG production by B cells. Thus, we have identified an essential role for DC-SIGN-induced ISGF3 by fucose-based PAMPs in driving IL-27 and subsequent T_{FH} polarization, which might be harnessed for vaccination design.

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Antibody-mediated responses are crucial to combat extracellular pathogens[1]. Humoural immunity is mediated by both type 2 T helper (T_H2) cells and follicular T helper (T_{FH}) cells that provide cognate help to B cells[2]. Establishment of protective long-term humoural immunity against extracellular pathogens such as parasites depends on the generation of high-affinity antibodies, a process that relies on formation of follicular structures called germinal centres (GCs) during infection. T_{FH} cells form a specialized subset of effector $CD4^+$ T cells required for GC formation. Once formed, T_{FH} cells maintain GCs and regulate the differentiation of GC B cells into memory B cells and long-lived plasma cells. T_{FH} cells produce interleukin (IL)-21, a potent cytokine that drives antibody isotype class switch recombination and B-cell proliferation, together with other factors[2,3]. Once B cells are outside GCs, IL-4 produced by T_H2 cells induces isotype switching to immunoglobulin E (IgE) antibodies[4–6], which are integral in eosinophil-mediated killing and expulsion of parasitic worms from the intestines[1,7]. Sequential switching of IgG cells to IgE is essential for the development of long-lived high-affinity IgE^+ plasma cells[8].

T helper cell (T_H) differentiation is under the control of dendritic cells (DCs) that sense invading pathogens through pattern recognition receptors, which triggers innate signalling that drives T_H polarization[9,10]. The factors involved in T_{FH} polarization, including the role of DCs, are not clear. Initial T_{FH} generation is thought to be driven by DCs, whereas B cells provide signals that reinforce the T_{FH} phenotype and/or promote their survival[3,11]. IL-6 and IL-21 have important roles *in vivo* in the generation and maintenance of T_{FH} cells and GC formation in mice, although conflicting data exist on their importance and redundancy in instructing T_{FH} differentiation[12–18]. Since IL-21 is produced by activated T cells, it was proposed that IL-6 produced by DCs initially induces expression of transcription factor Bcl-6 (refs 13,15), which acts as the master regulator of T_{FH} differentiation, including induction of IL-21 and chemokine receptor CXCR5, which is important for T_{FH} homing to B-cell zones and GC formation[2,14]. Data on the requirements for human T_{FH} differentiation are even more limited.

We have previously shown that triggering of dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), a pattern recognition receptor from the family of C-type lectin receptors, modulates T_H differentiation in a carbohydrate-specific manner[19,20]. The cytoplasmic tail of DC-SIGN is constitutively attached to adaptor protein LSP1 and the CNK-KSR1-Raf-1 signalosome, which are involved in downstream signalling when mannose-carrying ligands are recognized by DC-SIGN, leading to enhanced production of proinflammatory cytokines and T_H1 responses[19]. In contrast, priming of DCs by fucose-based glycan pathogen-associated molecular patterns (PAMPs) results in T_H2 differentiation[20]. Fucose-specific DC-SIGN signalling depends on crosstalk with Toll-like receptor (TLR) signalling and leads to activation of kinase IKKε in a LSP1-dependent but CNK-KSR1-Raf-1-independent manner. Subsequently, deactivation of deubiquitinase CYLD by IKKε-mediated phosphorylation leads to accumulation of ubiquitinated Bcl3 within the nucleus, where it modulates cytokine and chemokine expression by activated DCs to drive T_H2 polarization[20]. It is unknown whether DC-SIGN triggering also primes DCs for T_{FH} polarization.

Here we demonstrate that DC-SIGN triggering by fucose as well as fucose-carrying PAMPs from parasitic pathogens induces a specific innate signalling pathway that directs differentiation of T cells into Bcl-6^+CXCR5^+PD-1^{hi}Foxp1^{lo} T_{FH} cells by DCs via IFN-stimulated gene factor 3 (ISGF3)-induced IL-27 expression. Fucose-specific DC-SIGN signalling activates kinase IKKε and crosstalk between IKKε and interferon-α/β receptor (IFNR) signalling—which is activated as a result of TLR-induced type I IFN synthesis—and promotes assembly of ISGF3, a multimeric transcriptional activation complex composed of STAT1, STAT2 and IRF9. ISGF3 positively regulates expression of IL-27 subunit IL-27p28 via recruitment of RNA polymerase II (RNAP2), enhancing the *IL-27* transcription rate. DC-SIGN-dependent upregulation of IL-27 expression by DCs is crucial to the induction of Bcl-6^+ T_{FH} cells that secrete IL-21 and functionally induce IgG production by B cells. Thus, our data reveal an important role for DC-SIGN-IKKε signalling via crosstalk with IFNR signalling in priming DCs for T_{FH} polarization. Furthermore, we have identified IL-27 as important for human T_{FH} generation and the induction of humoural immunity to fucose-carrying extracellular parasitic pathogens.

Results

Fucose-specific DC-SIGN signalling leads to T_{FH} polarization. We investigated whether fucose-specific DC-SIGN signalling contributes to T_{FH} differentiation. DCs were primed with lipopolysaccharide (LPS) and/or Lewis X (LeX), and cocultured with naive $CD4^+$ T cells. After restimulation, we measured intracellular expression of T_{FH} 'master' transcription factor Bcl-6 (ref. 14). T_{FH} cells that induce GC formation and provide B-cell help *in vivo* are further characterized by high surface expression of receptors CXCR5 and PD-1, as well as secretion of IL-21 and low expression of transcription factor Foxp1, a negative regulator of IL-21 expression[2,3,17,21–24]. Strikingly, LPS/LeX-primed DCs induced strong expression of Bcl-6 in differentiated T_H cells; we measured $12.0 \pm 3.9\%$ ($n = 11$, $P = 0.0001$, Student's *t*-test) Bcl-6^+ T_H cells 24 h after restimulation (Fig. 1a). These *in vitro* generated Bcl-6^+ T_H cells also stained positive for CXCR5, while PD-1 expression was high and intracellular Foxp1 expression low (Fig. 1b), similar to *in vivo* generated T_{FH} cells. Bcl-6^- cells were both negative and positive for CXCR5 expression, showed low-to-intermediate PD-1 and high Foxp1 expression (Fig. 1c), most likely representing T_H1 and T_H2 populations, with CXCR5 known to be expressed on T_H2 cells[21]. Induction of T_{FH} cells was dependent on DC-SIGN signalling as DC priming in the presence of blocking DC-SIGN antibodies completely abrogated Bcl-6^+ T_H differentiation (Fig. 1a,c). Our naive T-cell pool contained no Bcl-6^+ cells, indicating that DC-SIGN-primed DCs induced T_H differentiation and not just proliferation. Neither immature DCs (iDCs), LeX-primed DCs nor LPS-primed DCs induced any Bcl-6-expressing T_H cells (Fig. 1a,c). T_{FH} differentiation was already optimal at 24 h after restimulation (Fig. 1c). We confirmed induction of Bcl-6 expression at the mRNA level in differentiated T_H cells by LPS/LeX-primed DCs, but neither by iDCs nor by LPS-primed DCs, in a DC-SIGN-dependent manner (Fig. 1d). Bcl-6 activation in T_{FH} cells is crucial for expression of IL-21, the main effector cytokine of T_{FH} cells[13–15]. DC-SIGN-dependent T_{FH} differentiation induced by DCs primed with LPS/LeX coincided with strong induction of IL-21 expression at both the mRNA and protein level, while LPS-primed DCs only induced minimal IL-21 expression by differentiated T_H cells (Fig. 1e,f). IL-21 can also be secreted by T_H-17 cells[25]; however, neither LPS- nor LPS/LeX-primed DCs induced T_H-17 differentiation as determined by mRNA expression of T_H-17 'master' regulator RORγt[25] (Supplementary Fig. 1), confirming that T_{FH} cells are the only source of IL-21 after T_H differentiation by LPS/LeX-primed DCs. Thus, triggering of TLR4 and fucose-specific DC-SIGN signalling specifically primes DCs to induce Bcl-6^+CXCR5^+PD-1^{hi}Foxp1^{lo} T_{FH} polarization.

We next investigated whether fucose-based PAMPs also induced T_{FH} differentiation. Soluble egg antigen (SEA) derived from helminth *Schistosoma mansoni* express fucose-containing LeX and LDN-F structures, whereas adult *Fasciola hepatica*

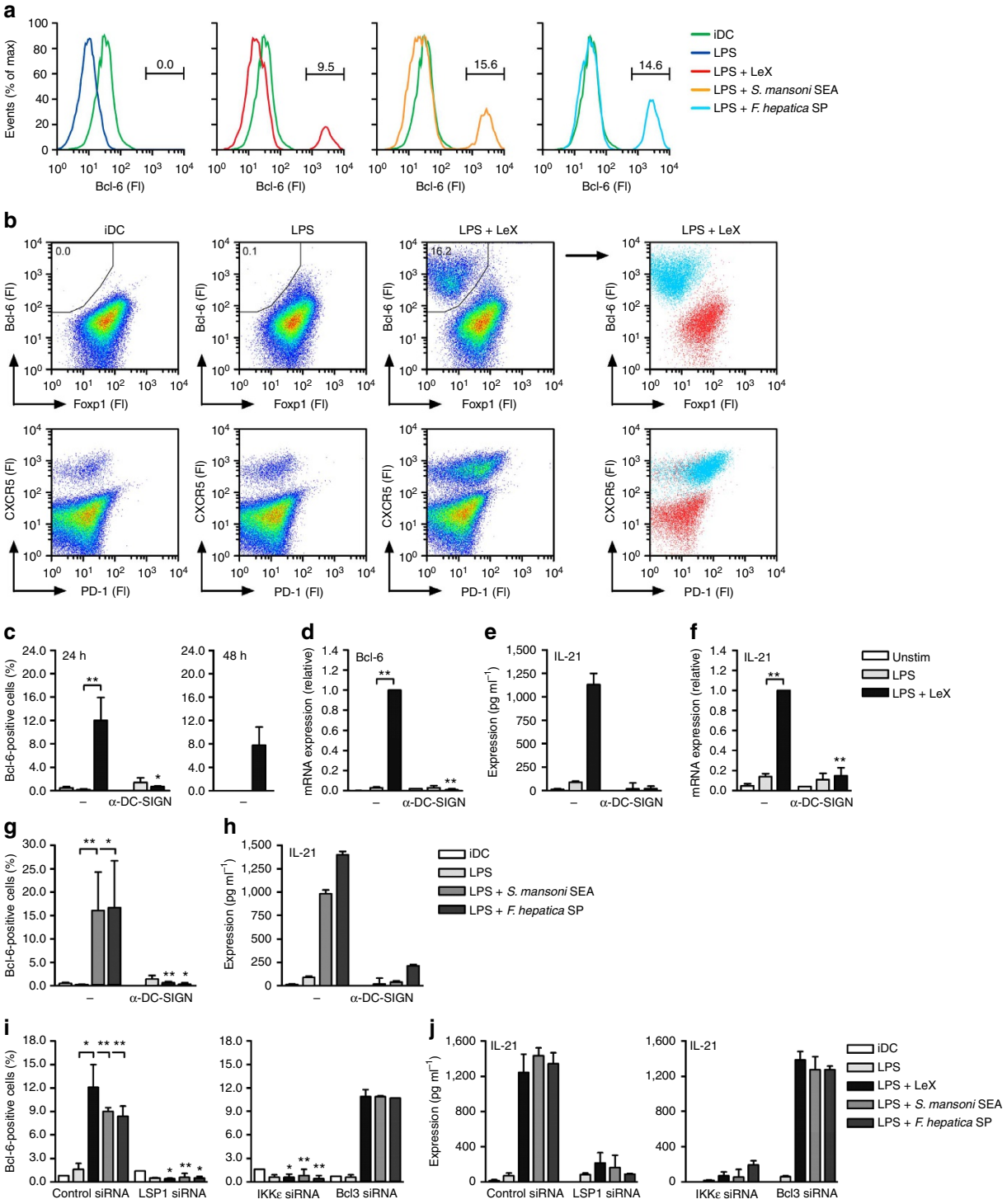


Figure 1 | DC-SIGN signalling by fucose-based PAMPs leads to IL-21-producing Bcl-6$^+$ T_{FH} development. (**a**,**c**,**g**,**i**) T_H polarization was determined by flow cytometry (FI, fluorescence intensity) 24 (**a**,**g**,**i**) or 48 (**c**) hours after restimulation by staining for intracellular Bcl-6 (T_{FH}) expression after coculture of naive CD4$^+$ T cells with immature DCs (iDCs) or DCs primed with LPS plus LeX, *S. mansoni* soluble egg antigen (SEA) or *F. hepatica* soluble products (SP), in the absence or presence of blocking DC-SIGN antibodies (**c**,**g**), or after LSP1, IKKε or Bcl3 silencing by RNA interference (siRNA) (**i**). Numbers above marker (**a**) indicate % Bcl-6$^+$ cells. $^{**}P<0.01$, $^{*}P<0.05$ (Student's *t*-test). (**b**) Bcl-6, CXCR5, PD-1 and Foxp1 expression was determined by flow cytometry 24 h after restimulation, after coculture of naive CD4$^+$ T cells with iDCs or DCs primed with LPS plus LeX. Numbers in gate indicate % Bcl-6$^+$ cells. Bcl-6$^+$ cells are represented in cyan and Bcl-6$^-$ cells in red in right panels. (**d**,**f**) Bcl-6 (**d**) and IL-21 (**f**) mRNA expression by differentiated T cells 24 h after restimulation, after coculture with iDCs or DCs primed with LPS plus LeX, in the absence or presence of blocking DC-SIGN antibodies, measured by real-time PCR, normalized to GAPDH and set at 1 in differentiated T cells cocultured with LPS/LeX-primed DCs. $^{**}P<0.01$, $^{*}P<0.05$ (Student's *t*-test). (**e**,**h**,**j**) IL-21 secretion in supernatants of differentiated T cells 24 h after restimulation, after coculture with iDCs or DCs primed with LPS plus LeX, *S. mansoni* SEA or *F. hepatica* SP, in the absence or presence of blocking DC-SIGN antibodies (**e**,**h**), or after LSP1, IKKε or Bcl3 silencing (**j**) was measured by ELISA. Data are representative of at least eleven (**a**, LeX; **c**, 24 h without block), five (**a**, SEA and SP; **g**, 24 h without block), two (**b**), three (**c**,**g**, 24 h with block; **c**, 48 h; **i**,**j**), nine (**d**,**f**) or four (**e**,**h**) independent experiments (mean ± s.d. in **c**,**d**,**f**,**g**,**i**; mean ± s.d. of duplicates in **e**,**h**,**j**). Unstim, unstimulated.

flatworms express LDN-F[26] that mediate interactions with DC-SIGN[27] (Supplementary Fig. 2). DCs primed with LPS plus either *S. mansoni* SEA or *F. hepatica* soluble products (SP) also induced T_{FH} differentiation as determined by percentages of Bcl-6$^+$ cells and Bcl-6 mRNA expression (Fig. 1a,f and Supplementary Fig. 3). Again, Bcl-6$^+$ T_H cells were further characterized as CXCR5$^+$, PD-1^{hi} and Foxp1^{lo} (Supplementary Fig. 3). DCs primed with *S. mansoni* SEA or *F. hepatica* SP alone, similar to LeX alone, did not induce any T_H proliferation. Moreover, DC priming with LPS plus either *S. mansoni* SEA or *F. hepatica* SP induced strong IL-21 secretion by differentiated T_H cells, which was dependent on DC-SIGN signalling (Fig. 1g and Supplementary Fig. 3), indicating that different fucose-based PAMPs can prime DCs via DC-SIGN to induce T_{FH} polarization.

We previously identified a fucose-specific DC-SIGN signalling pathway that primes TLR-stimulated DCs via LSP1-dependent IKKε-mediated Bcl3 activation to induce T_H2 responses[20]. We investigated whether this signalling pathway is similarly crucial for T_{FH} differentiation. Both LSP1 and IKKε silencing in DCs by RNA interference (Supplementary Fig. 4) inhibited T_{FH} polarization by DCs primed with LPS plus either LeX, *S. mansoni* SEA or *F. hepatica* SP (Fig. 1h,i). Strikingly, T_{FH} polarization by Bcl3-silenced LPS/LeX-primed DCs was unaffected and resulted in similar percentages of Bcl-6$^+$ T_{FH} cells and IL-21 production as compared with differentiation in response to control-silenced primed DCs (Fig. 1h,i). Thus, fucose engagement of DC-SIGN induces LSP1-IKKε-dependent but Bcl3-independent signalling that modulates TLR-induced priming of DCs, which is crucial for T_{FH} polarization.

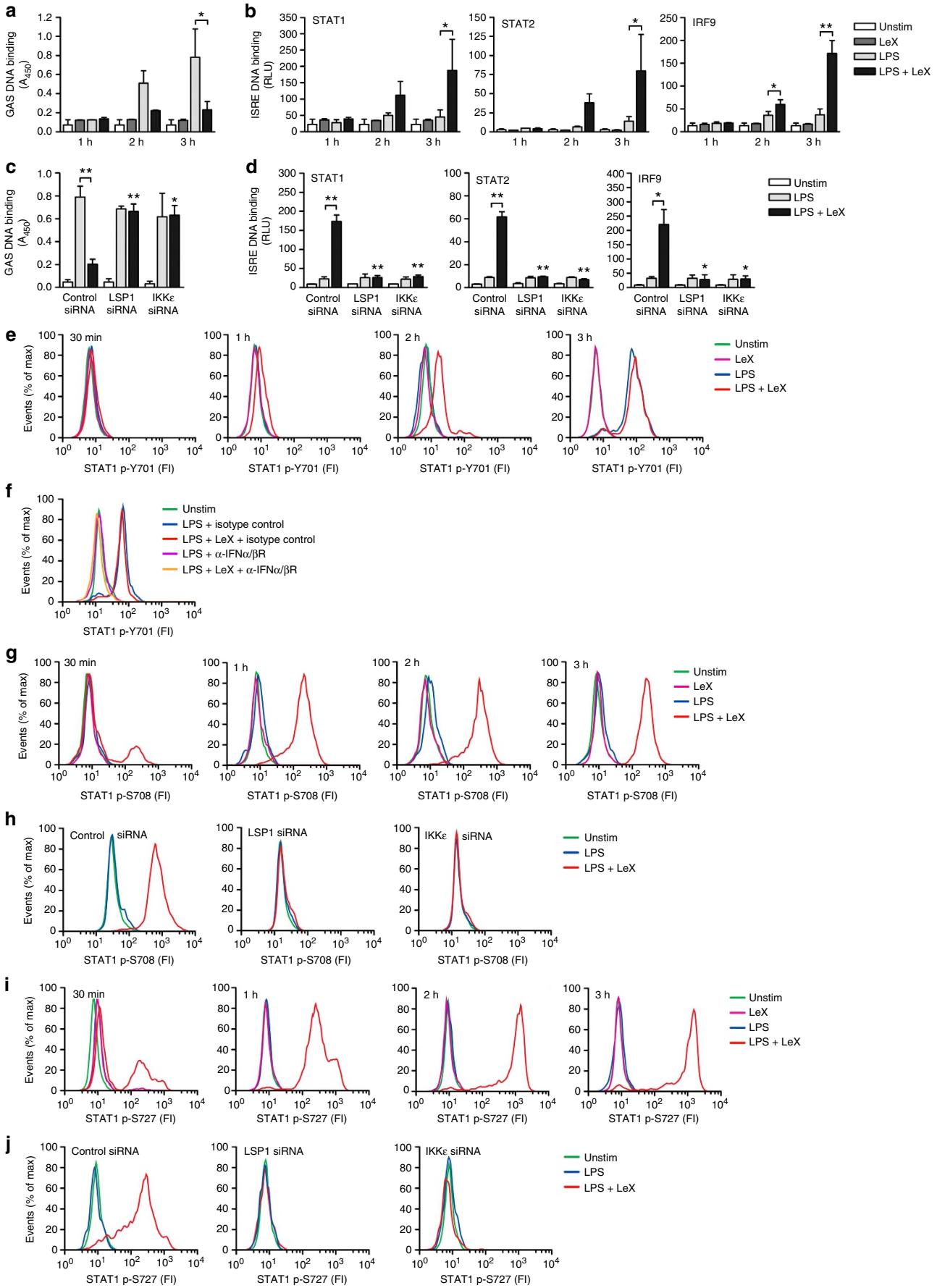
Crosstalk between DC-SIGN and IFNR signalling activates ISGF3. To elucidate the mechanism behind DC-SIGN-IKKε-dependent priming of DCs for T_{FH} differentiation, we considered different effectors of IKKε, besides CYLD[20]. Transcription factor STAT1 is a target for IKKε that induces phosphorylation at Ser708 (ref. 28). Ser708 phosphorylation of STAT1 is suggested to inhibit STAT1 homodimerization without disrupting STAT1–STAT2 dimerization[29], which precedes recruitment of DNA binding unit IRF9 to form ISGF3 (ref. 30). Therefore, we investigated activation of STAT1 homodimers and ISGF3 after DC-SIGN triggering. We found that LPS stimulation of DCs induced nuclear translocation of STAT1 homodimers as determined by binding to IFN-γ-activated sites (GAS)[30] (Fig. 2a). Strikingly, LeX triggering of DC-SIGN largely inhibited LPS-induced STAT1–STAT1 binding to GAS sites (Fig. 2a), while strongly inducing ISGF3 formation, which bound to IFN-stimulated response elements (ISRE)[30] (Fig. 2b). Silencing of LSP1 or IKKε abrogated ISGF3 formation after DC-SIGN triggering (Fig. 2d) and led to induction of STAT1 homodimers (Fig. 2c). These data therefore indicate that DC-SIGN signalling via IKKε promotes ISGF3 formation, while inhibiting STAT1 homodimerization.

Formation of STAT1 homodimers as well as ISGF3 requires STAT1 phosphorylation at Tyr701, which controls dimerization of STAT1 with either STAT1 or STAT2, and is a prerequisite for nuclear translocation[30,31]. STAT1 phosphorylation at Tyr701 is driven by IFNR signalling and in the case of TLR triggering requires *de novo* synthesis of type I IFNs[30,32]. Indeed, TLR-induced IFNR-mediated STAT1 phosphorylation at Tyr701 was detected after 2 h and further increased after 3 h of stimulation (Fig. 2e,f and Supplementary Fig. 5), which corresponded with GAS binding activity (Fig. 2a,b), and was not affected by DC-SIGN triggering. We next addressed whether DC-SIGN signalling directs the observed specific ISGF3 formation via STAT1 phosphorylation at Ser708. We detected efficient STAT1 phosphorylation at Ser708 after 30 min of stimulation with LPS/LeX (Fig. 2g), thus preceding IFNR-induced Tyr701 phosphorylation (Fig. 2e), whereas neither LPS nor LeX alone induced Ser708 phosphorylation (Fig. 2g). STAT1 Ser708 phosphorylation induced by LPS/LeX further increased over time (Fig. 2g and Supplementary Fig. 5) in a LSP1- and IKKε-dependent manner (Fig. 2h). Notably, we also detected LSP1- and IKKε-dependent phosphorylation of STAT1 at Ser727 (Fig. 2i,j and Supplementary Fig. 5), a site that resides in the transactivation domain of STAT1 and strongly enhances its transactivation potential[33]. Like Ser708 phosphorylation, IKKε-mediated Ser727 phosphorylation was irrespective of IFNR signalling (Supplementary Fig. 5). These data indicate that DC-SIGN-IKKε-dependent signalling directs preferential formation of ISGF3 via STAT1 phosphorylation at Ser708, while enhancing ISGF3 transactivation activity via Ser727 phosphorylation.

Both STAT1 homodimers and ISGF3 are involved in an autocrine positive feedback loop to boost type I IFN expression via induction of IFN-stimulated genes (ISGs)[34]. We next investigated whether DC-SIGN signalling enhanced type I IFN responses through ISGF3. LPS induced IFN-β mRNA expression early after 2 h as well as a second peak at 6 h post stimulation (Fig. 3a), which was dependent on IFNR signalling (Fig. 3b). LPS/LeX costimulation significantly enhanced and prolonged late IFN-β expression, which depended on DC-SIGN-induced IKKε activation and ISGF3 formation as determined by silencing of IKKε and IRF9, the DNA binding unit of ISGF3 (Fig. 3a). Transcription factor IRF7 is a known ISG as well as a regulator of IFN-β transcription[34]. DC-SIGN triggering significantly enhanced LPS-induced IFN-mediated IRF7 expression in an IKKε- and IRF9-dependent manner (Fig. 3c,d). Silencing of IRF7 abrogated the increased type I IFN responses, confirming that the DC-SIGN-mediated autocrine feedback loop on IFN-β expression was dependent on IRF7 expression (Fig. 3a). Expression of archetypical ISGs MxA and OAS1 (ref. 34) was also strongly enhanced after LPS/LeX costimulation (Supplementary Fig. 6). These data show that crosstalk between fucose-specific DC-SIGN signalling and IFNR signalling specifically activates ISGF3, which might regulate a gene programme that underlies DC-SIGN-dependent T_{FH} polarization by primed DCs.

Fucose-specific DC-SIGN signalling enhances IL-27 expression. We next set out to find the factor(s) that orchestrate DC-SIGN-dependent priming of DCs for T_{FH} differentiation. Type I IFN signalling in DCs has previously been reported to be involved in the development of T_{FH} cells in mice via the induction of IL-6 (ref. 35). We have previously shown that IL-6 expression is downregulated in response to TLR4 and fucose-specific DC-SIGN

Figure 2 | Fucose-specific DC-SIGN signalling induces STAT1 phosphorylation at Ser708 and Ser727 via IKKε, thereby favouring ISGF3. (**a**–**d**) STAT1 homodimer and ISGF3 (STAT1–STAT2–IRF9 complex) activation in nuclear extracts of DCs 1, 2 or 3 (**c**,**d**) hours after stimulation with LPS and/or LeX, after LSP1 or IKKε silencing, measured by GAS (**a**,**c**) or ISRE (**b**,**d**) DNA binding ELISA, respectively. RLU, relative light units. $^{**}P<0.01$, $^{*}P<0.05$ (Student's *t*-test). (**e**–**j**) STAT1 phosphorylation at Tyr701 (**e**,**f**), Ser708 (**g**,**h**) or Ser727 (**i**,**j**) in DCs left unstimulated (Unstim; green) or 30 min, 1, 2 or 3 h (**f**,**h**,**j**) after stimulation with LPS (blue), LeX (pink) or LPS/LeX (red), in the presence of isotype control (LPS, purple; LPS/LeX, orange) or blocking IFNR antibodies (**f**) or after LSP1 or IKKε silencing (**h**,**j**), determined by flow cytometry (FI, fluorescence intensity). Data are representative of four (**a**,**b**,**e**,**f**,**j**), three (**c**,**d**,**h**) or five (**g**,**i**) independent experiments (mean ± s.d. in **a**–**d**).



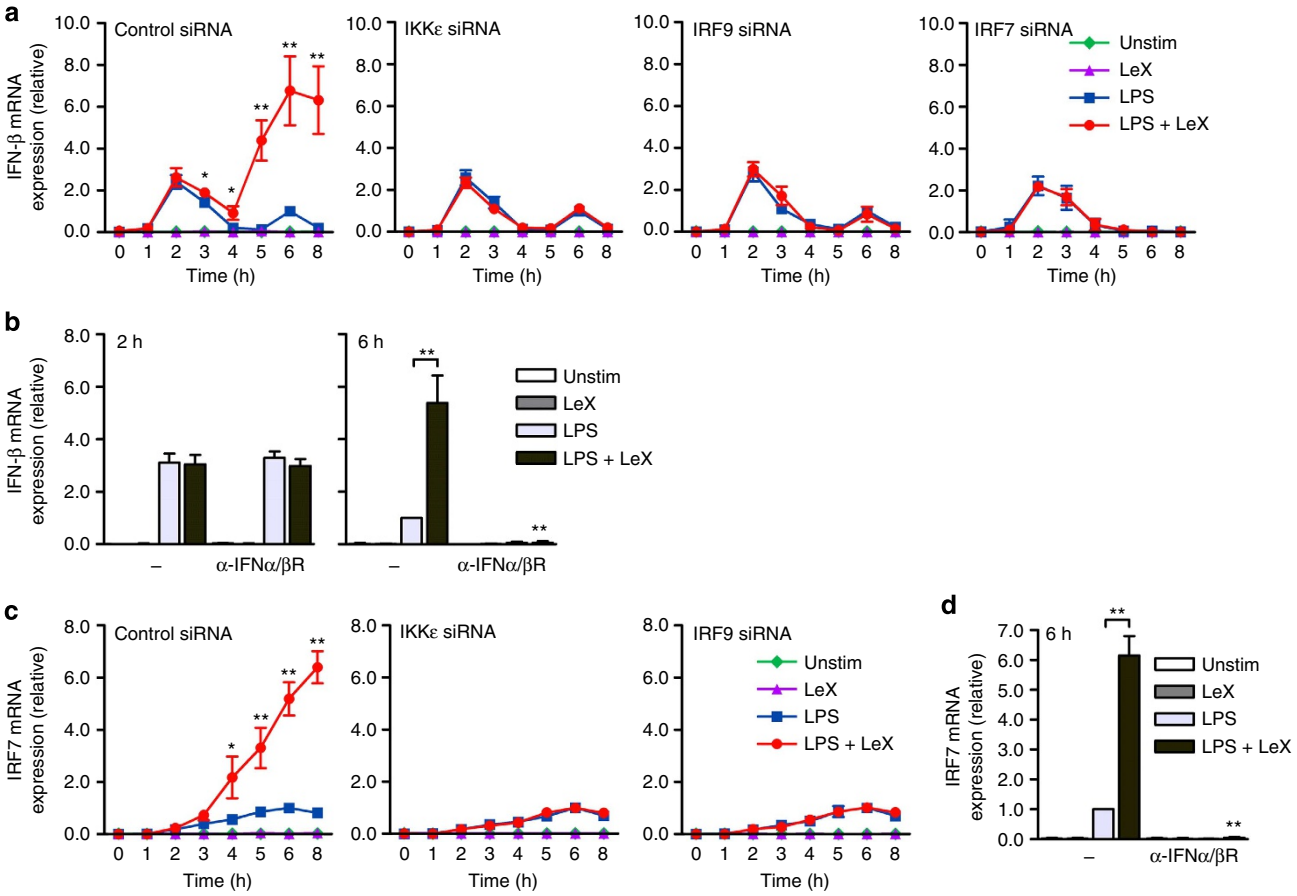


Figure 3 | Fucose-specific DC-SIGN signalling enhances type I IFN responses via ISGF3-dependent IRF7 expression. (**a**–**d**) IFN-β (**a**,**b**) and IRF7 (**c**,**d**) mRNA expression by DCs at indicated times after stimulation with LPS and/or LeX, after IKKε (**a**,**c**), IRF9 (**a**,**c**) or IRF7 (**a**) silencing, or in the absence or presence of blocking IFNR antibodies (**b**,**d**), measured by real-time PCR, normalized to GAPDH and set at 1 in 6 h LPS-stimulated cells. $^{**}P<0.01$, $^{*}P<0.05$ (Student's *t*-test). Data are representative of at least four (**a**,**c**) or five (**b**,**d**) independent experiments (mean ± s.d.). Unstim, unstimulated.

costimulation via a DC-SIGN-induced IKKε-Bcl3 pathway[20]. Here we found that IL-6 expression in response to LPS/LeX stimulation was independent of IFNR triggering or ISGF3 activation, as determined by blocking IFNR antibodies and IRF9 silencing, respectively (Fig. 4a and Supplementary Fig. 7), suggesting that IL-6 is not the crucial factor that underlies DC-SIGN-dependent T_{FH} polarization by LPS/LeX-primed DCs.

Since T_{FH} generation is impaired in mice lacking IL-27R (ref. 36) and the promoter region of *IL-27*, which encodes cytokine subunit IL-27p28, contains a functional ISRE[37], we investigated IL-27 expression by DC-SIGN. Strikingly, LeX stimulation enhanced LPS-induced IL-27 expression by DCs several fold in a DC-SIGN-dependent manner (Fig. 4b). LeX, *S. mansoni* SEA and *F. hepatica* SP alone did not induce IL-27 (Fig. 4b,c). IL-27 is a heterodimeric cytokine composed of IL-27p28 and Epstein-Barr virus-induced gene 3 (EBI3) subunits[38]. We found that IL-27 protein expression was modulated by DC-SIGN signalling at the level of IL-27p28 transcription; while both IL-27p28 and IL-27EBI3 mRNA expression was induced by LPS alone, LeX costimulation enhanced IL-27p28 mRNA levels approximately threefold (2.6 ± 0.27, $n=10$, $P<0.001$, Student's *t*-test) in a DC-SIGN- and IFNR-dependent manner, whereas it did not affect *EBI3* transcription (Fig. 4d). Furthermore, both LSP1 and IKKε silencing abrogated LeX-induced increase of IL-27p28 mRNA expression after LPS stimulation (Fig. 4e). In addition, LeX increased IL-27p28 mRNA expression after TLR3 stimulation with poly(I:C), but not after TLR2 triggering with Pam3CSK4 (Fig. 4f). This reflects the inability of TLR2 but not TLR3 to induce IFN-β synthesis (Supplementary Fig. 8), which is required for crosstalk with DC-SIGN signalling to induce ISGF3 formation and as such for ISGF3-dependent IL-27p28 upregulation (Fig. 4d). Parasites such as *S. mansoni* carry or secrete TLR3 and TLR4 ligands[39,40] that makes IFNR- and DC-SIGN-driven ISGF3 formation during parasite infections physiologically relevant. Interfering with DC-SIGN signalling via blocking DC-SIGN antibodies, LSP1 or IKKε silencing completely abrogated IL-27p28 upregulation by parasitic PAMPs (Fig. 4g,h). As expected, Bcl3 silencing had no effect on LeX modulation of LPS-induced IL-27p28 mRNA expression (Fig. 4e,h). These data show that DC-SIGN triggering affects IL-27 expression via a LSP1- and IKKε-dependent increase in IL-27p28 expression.

IKKε-dependent ISGF3 activation enhances *IL-27* transcription. To elucidate the role of ISGF3 in IL-27p28 expression in response to DC-SIGN signalling, we performed chromatin immunoprecipitation (ChIP) assays. We observed that both STAT1 and IRF9 were bound to the ISRE within the *IL-27* promoter after LPS/LeX stimulation (Fig. 5a). To establish whether STAT1 binds as part of ISGF3 to the *IL-27* ISRE, we performed re-ChIP experiments in which we performed a second immunoprecipitation with STAT2 or IRF9 antibodies on DNA–STAT1 complexes. We detected both STAT1–STAT2 and STAT1–IRF9 complexes bound to the *IL-27* promoter after LPS/LeX stimulation (Fig. 5b). Silencing of IRF9

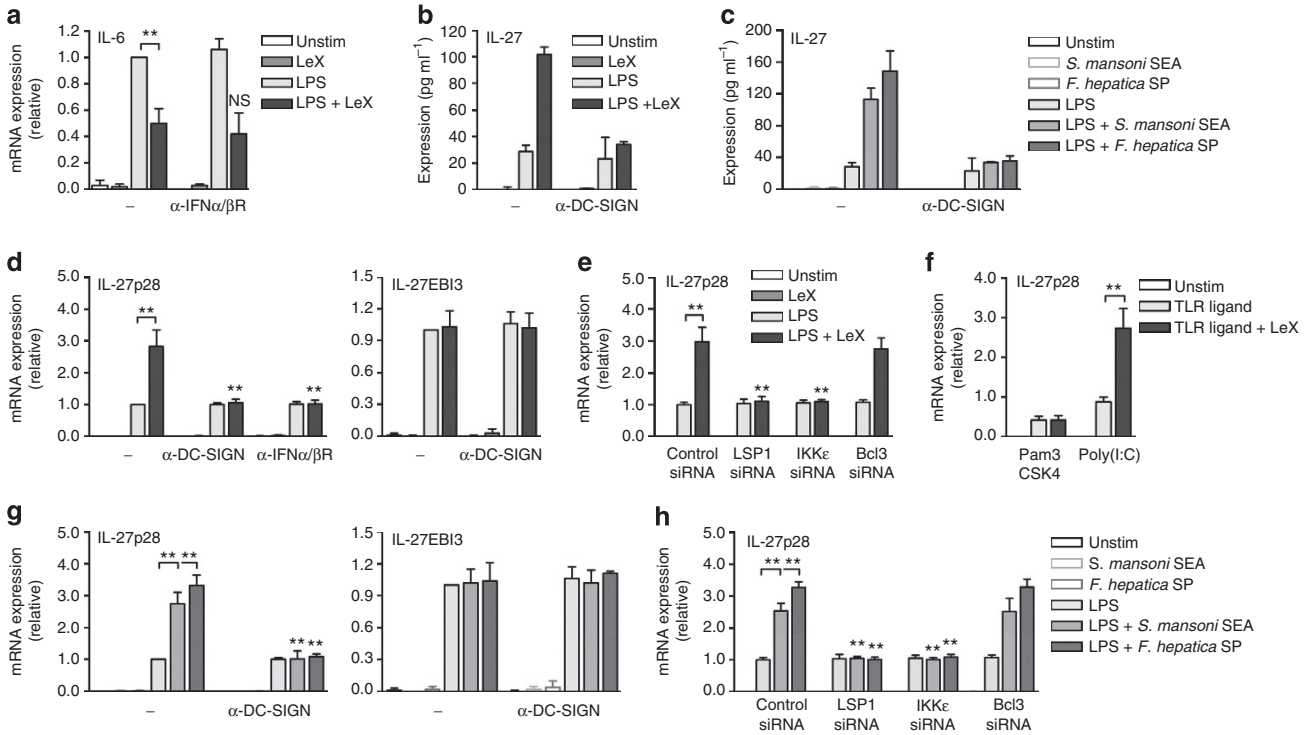


Figure 4 | Fucose-specific DC-SIGN signalling specifically upregulates IL-27p28 via IKKε. (**a**,**d**–**h**) IL-6 (**a**), IL-27p28 (**d**–**h**) and IL-27EBI3 (**d**,**g**) mRNA expression by DCs 6 hours after stimulation with LPS, PamCSK3 or poly(I:C) and/or LeX, *S. mansoni* SEA or *F. hepatica* SP, in the absence or presence of blocking IFNR (**a**,**d**) or DC-SIGN (**d**,**g**) antibodies, or after LSP1, IKKε or Bcl3 silencing (**e**,**g**), measured by real-time PCR, normalized to GAPDH and set at 1 in TLR-stimulated cells. $^{**}P<0.01$, NS, not significant (Student's *t*-test). (**b**,**c**) IL-27 secretion in supernatants of DCs 24 h after stimulation with LPS and/or LeX, *S. mansoni* SEA or *F. hepatica* SP, in the absence or presence of blocking DC-SIGN antibodies. Data are representative of at least eight (**a**), four (**b**,**c**,**g**,**h**), five (**d**), nine (**e**) or seven (**f**) independent experiments (mean ± s.d. in **a**,**d**–**h**; mean ± s.d. of duplicates in **b**,**c**). Unstim, unstimulated.

completely abrogated STAT1 recruitment to the *IL-27* ISRE (Fig. 5c), indicating that all STAT1 bound to *IL-27* is part of ISGF3 complexes. ISGF3 binding could already be detected 2 h post stimulation and further increased after 3 h of LPS/LeX stimulation (Fig. 5c), which follows the nuclear appearance of ISGF3 (Fig. 2b). Moreover, LSP1 and IKKε silencing abrogated binding of ISGF3 to *IL-27* (Fig. 5c). These results show that DC-SIGN signalling via IKKε results in ISGF3 recruitment to the *IL-27* promoter.

Recent studies have demonstrated that ISGF3 recruits RNAP2 to ISRE-responsive promoters[41]. Further ChIP analyses showed that LPS induced low levels of RNAP2 recruitment to *IL-27*, which was strongly enhanced by LeX (Fig. 5d). This increase in recruitment of RNAP2 followed ISGF3 binding to the *IL-27* promoter in time and was completely abolished by IRF9 silencing (Fig. 5d). These data strongly indicate that DC-SIGN signalling increases transcriptional induction of *IL-27* via ISGF3-facilitated recruitment of RNAP2. Therefore, we determined the effect of DC-SIGN on the transcription rate from the *IL-27* gene at different time points. After 1 h of stimulation, we detected similar *IL-27* transcription rates in LPS- and LPS/LeX-stimulated DCs (Fig. 5e). However, 3 h post stimulation, LeX enhanced the LPS-induced *IL-27* transcription rate approximately fivefold (4.97 ± 0.68, $n=4$, $P=0.001$, Student's *t*-test; Fig. 5e). IRF9 silencing completely abrogated this increase in transcription rate (Fig. 5e), demonstrating that ISGF3 activation is instrumental in DC-SIGN-induced upregulation of IL-27p28 expression via RNAP2 recruitment to the *IL-27* promoter. While LPS-induced expression of IL-27p28 showed transient mRNA accumulation that peaked at 6 h post stimulation, LPS/LeX stimulation significantly accelerated *IL-27* transcription from 3 h post stimulation, which further increased over time (Fig. 5f). IRF9 silencing completely blocked any increase in *IL-27* transcription in response to LeX (Fig. 5f), supporting our conclusion that ISGF3 activation is required for enhancement of IL-27 expression by DC-SIGN triggering via RNAP2 recruitment.

DC-SIGN-induced IL-27 expression directs T_{FH} polarization. To elucidate the contribution of DC-SIGN-induced IL-27 to T_{FH} polarization after DC priming, we neutralized IL-27-mediated effects in T_H differentiation assays during DC-T-cell coculture. Remarkably, neutralizing IL-27 antibodies completely abrogated T_{FH} polarization by LPS plus fucose-primed DCs (Fig. 6a–c). Similarly, induction of T_{FH} cells and IL-21 secretion by differentiated T_H cells in response to DCs primed with LPS plus *S. mansoni* SEA or *F. hepatica* SP was completely blocked by neutralizing IL-27 antibodies (Fig. 6a–c). Neutralizing IL-27 antibodies did not affect DC-SIGN-dependent T_H2 polarization by LPS/LeX-primed DCs (Supplementary Fig. 9). Vice versa, the addition of rhIL-27 to T cells cocultured with LPS-primed DCs induced T_{FH} polarization in the absence of LeX priming of DCs, indicating that a threshold level of IL-27—as induced after DC-SIGN triggering—is required for T_{FH} induction (Fig. 6d,e). In contrast, addition of rhIL-6 did not affect T_{FH} differentiation in response to LPS-primed DCs (Fig. 6d,e). Neutralizing IL-6 antibodies did partially block T_{FH} polarization in response to LPS/LeX-primed DCs (Fig. 6d,e). These results indicate that both IL-27 and IL-6 are required, but DC-SIGN-induced IL-27 provides the decisive signal for T_{FH} polarization. Moreover, blocking DC-SIGN-mediated ISGF3 activation and hence IL-27 expression via IRF9 silencing in DCs, similarly as neutralizing IL-27 antibodies, blocked Bcl-6 induction in and IL-21 secretion by differentiated T_H cells in response to

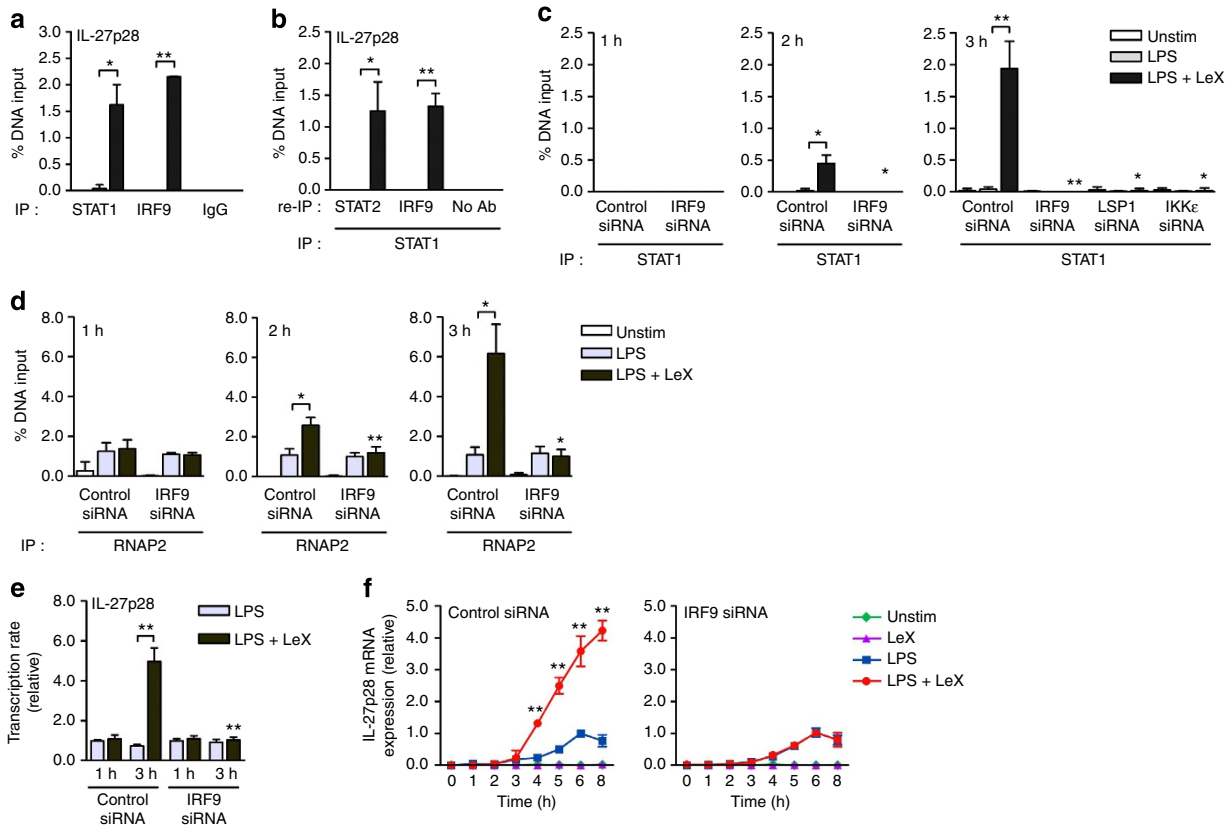


Figure 5 | IKKε-dependent ISGF3 activation accelerates *IL-27* transcription via RNAP2 recruitment. (**a**–**d**) STAT1 (**a**,**c**), IRF9 (**a**), STAT1–STAT2 and STAT1–IRF9 (ISGF3; **b**) and RNA polymerase II (RNAP2) (**d**) recruitment to ISRE binding motif (**a**–**c**) and TATA box (**d**) of the *IL-27* promoter in DCs at indicated times (3 hours in **a**,**b**) after stimulation with LPS and/or LeX, after IRF9, LSP1 or IKKε silencing, determined by ChIP (**a**,**c**,**d**) and re-ChIP (**b**) assay. IgG and no antibody (Ab) indicate negative controls. Data are expressed as % input DNA. $^{**}P<0.01$, $^{*}P<0.05$ (Student's *t*-test). (**e**) *IL-27* transcription rate in DCs at indicated times after stimulation with LPS and/or LeX, after IRF9 silencing, determined by nuclear run-on assay, normalized to β-actin and set at 1 in 1 h LPS-stimulated control-silenced DCs. $^{**}P<0.01$ (Student's *t*-test). (**f**) IL-27p28 mRNA expression by DCs over time after stimulation with LPS and/or LeX, after IRF9 silencing, measured by real-time PCR, normalized to GAPDH and set at 1 in 6 h LPS-stimulated cells. $^{**}P<0.01$ (Student's *t*-test). Data are representative of at least four (**a**,**e**,**f**) or three (**b**–**d**) independent experiments (mean ± s.d.). IP, immunoprecipitation; Unstim, unstimulated.

LPS plus fucose-primed DCs (Fig. 6f,g), without affecting T_H2 polarization (Supplementary Fig. 9). Thus, DC-SIGN-induced expression of IL-27 is crucial to priming DCs for T_{FH} polarization.

T_{FH} cells promote B-cell IgG production and survival. T_{FH} cells provide cognate help to B cells to ensure long-lived humoural immunity after infection[2,3]. We performed B-cell help assays to establish whether T_{FH} polarization via IL-27 in response to fucose-specific DC-SIGN-mediated priming of DCs induces antibody production by B cells. We isolated $CD19^+$ B cells from peripheral blood containing both IgM-producing naive B cells and isotype-committed memory B cells. Next, we cocultured these B cells with T_H cells differentiated by differently primed DCs and after T-cell-specific restimulation, we measured IgM and IgG production by activated B cells. T_H cells differentiated by LPS-primed DCs induced production of both IgM and IgG by B cells (Fig. 7). However, IgG production was strongly induced and IgM production decreased after coculture of B cells with DCs primed with LPS plus fucose-based PAMPs (Fig. 7). Strikingly, blocking DC-SIGN-mediated T_H differentiation via neutralizing IL-27 antibodies during DC-T-cell coculture strongly diminished IgG production by activated B cells while leaving IgM production undisturbed (Fig. 7a). T_{FH} cells also promote B-cell survival in GCs via PD-1 signalling[42]. Annexin V/propidium iodide (PI) stainings showed that T_{FH} cells generated *in vitro* by DCs primed with fucose PAMPs also supported B-cell survival, in an IL-27-dependent manner (Fig. 7b). These results suggest that fucose-specific DC-SIGN-mediated priming of DCs is essential to the induction of T_{FH} cells and cognate B-cell help to induce IgG production.

Discussion

Induction of T_{FH} cells is essential for antibody-mediated immunity via GC formation and differentiation of memory B cells and plasma cells. Here we demonstrate that engagement of DC-SIGN by fucose-based PAMPs primes DCs for T_{FH} polarization. At the molecular level, we identified IKKε as the crucial conductor for T_{FH} differentiation (Supplementary Fig. 10). Crosstalk between fucose-specific DC-SIGN and type I IFNR signalling favoured formation of ISGF3 complexes consisting of STAT1–STAT2 and IRF9 over STAT1 homodimerization owing to IKKε-mediated STAT1 phosphorylation at Ser708. ISGF3 increased the transcription rate of the *IL-27* gene, encoding the IL-27p28 subunit, by recruiting RNAP2 to its promoter. We found that the increase in IL-27 production was crucial for the development of T_{FH} cells and T-cell help to B cells for the

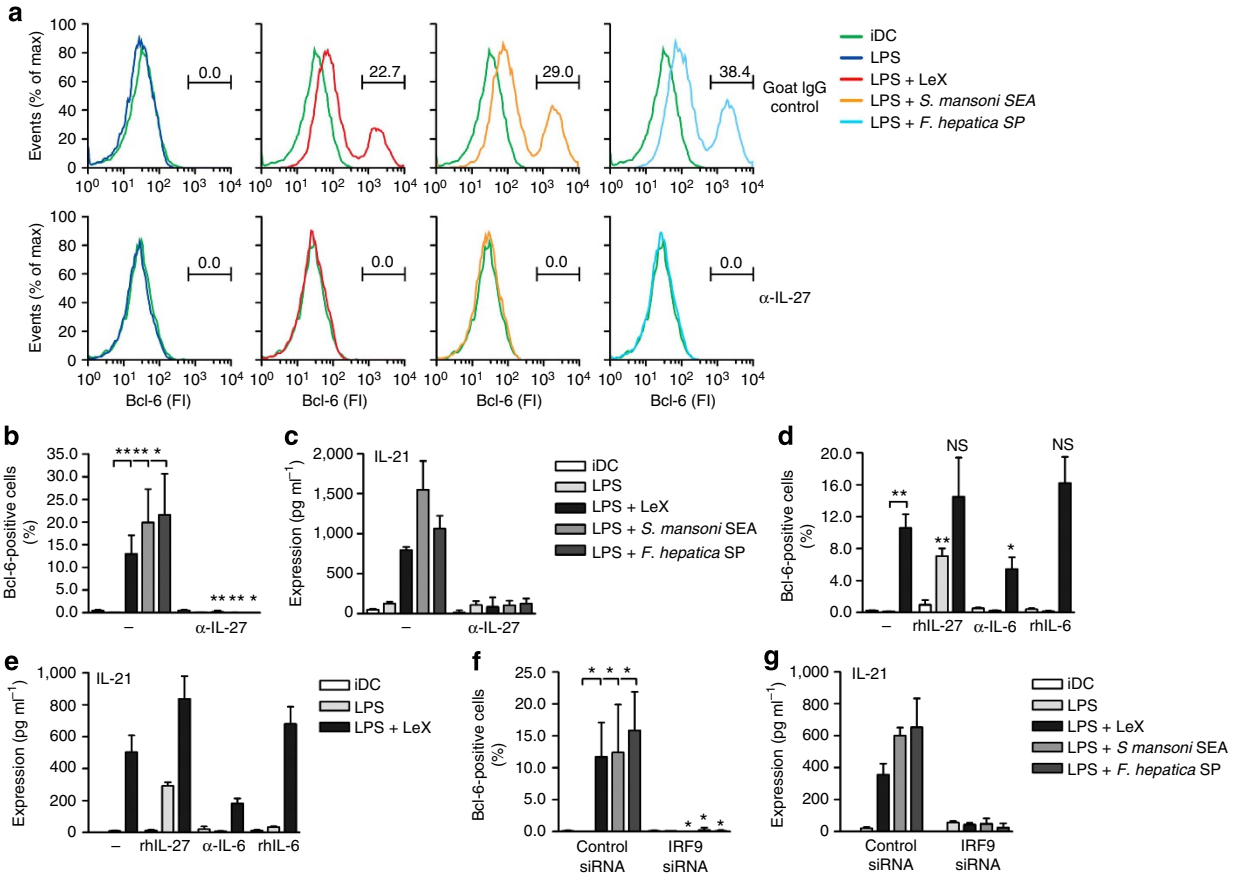


Figure 6 | DC-SIGN-induced IL-27 expression is crucial for T_{FH} polarization. (**a**,**b**,**d**,**f**) T_H polarization was determined by flow cytometry (FI, fluorescence intensity), 24 hours after restimulation, by staining for intracellular Bcl-6 (T_{FH}) expression after coculture of naive $CD4^+$ T cells with immature DCs (iDC) or DCs primed with LPS plus LeX, *S. mansoni* SEA or *F. hepatica* SP. IL-27 (**a**,**b**,**f**) or IL-6 (**d**) stimulation of T cells was inhibited by neutralizing IL-27 (**a**,**b**) or IL-6 (**d**) antibodies during DC-T-cell coculture, with normal goat IgG as control (**a**), or via IRF9 silencing of DCs (**f**). In **d**, DC-T cocultures were supplemented with rhIL-27 or rhIL-6. Numbers above marker (**a**) indicate % Bcl-6$^+$ cells. $^{**}P<0.01$, $^{*}P<0.05$, NS = not significant (Student's *t*-test). (**c**,**e**,**g**) IL-21 secretion in supernatants of differentiated T cells 24 h after restimulation, after coculture with iDCs or DCs primed with LPS plus LeX, *S. mansoni* SEA or *F. hepatica* SP, measured by ELISA. IL-27 and IL-6 effects on T cells were either blocked or stimulated as described above. Data are representative of nine (**a**,**c**, LeX), five (**a**,**c**, SEA, SP), eight (**b**) or four (**d**–**g**) independent experiments (mean ± s.d. in **b**,**d**,**f**; mean ± s.d. of duplicates in **c**,**e**,**g**).

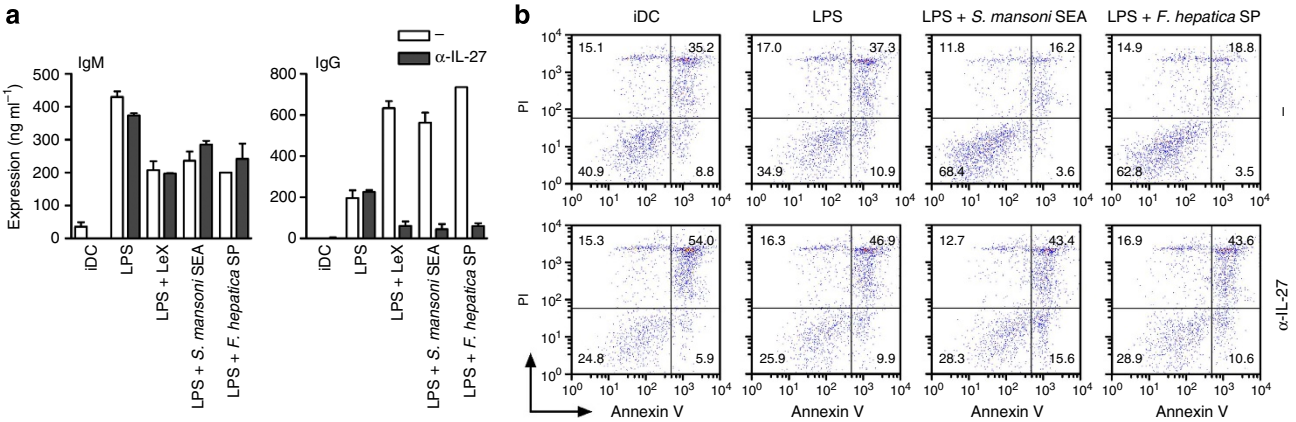


Figure 7 | DC-SIGN-induced IL-27 expression is crucial for B cells IgG production via T-cell help. (**a**) IgM and IgG secretion in supernatants of B cells 7 days after stimulation with T cells that were differentiated after coculture with immature DCs (iDCs) or DCs primed with LPS plus LeX, *S. mansoni* SEA or *F. hepatica* SP, measured by ELISA. IL-27 effects on T cells were blocked by neutralizing IL-27 antibodies during DC-T-cell coculture. (**b**) B-cell survival 48 hours after stimulation with T cells that were differentiated after coculture with iDCs or DCs primed with LPS plus *S. mansoni* SEA or *F. hepatica* SP was determined by flow cytometry via Annexin V-FITC/PI staining. Numbers in quadrants indicate % cells in each. Data are representative of three (**a**) or two (**b**) independent experiments (mean ± s.d. of duplicates in **a**).

production of IgG and survival, which might contribute to development of long-term humoural responses to, for example, parasites such as *S. mansoni* and *F. hepatica* that express various fucose-based glycan PAMPs[26].

The origin of T_{FH} cells and their relationship with other T_H subsets is the subject of ongoing investigations. New insights have led to a multistage, multifactorial model in which DCs initially prime T_{FH} differentiation; however, these signals are thought to be transient, and B cells are required at a second stage to sustain T_{FH} differentiation. In the absence of this second stage, T cells might revert and follow a different differentiation path depending on their unique microenvironments[2,43]. Our data show that fucose-primed DCs indeed induce T_{FH} differentiation from naive T cells. Our data also suggest that T_{FH} fate is established at the time of DC priming and develops exclusively of other T_H subsets that opposes observations in mice immunized with *S. mansoni* SEA, where T_{FH} cells seemed to convert from T_H2 precursor cells[44]; we observed that generation of T_{FH} and T_H2 subsets required different priming signals, as Bcl3 silencing in DCs did not affect T_{FH} differentiation, while IRF9 silencing or neutralization of IL-27 signals did not interfere with T_H2 differentiation.

We established the noncanonical kinase IKKε as the central player in T_H polarization via DC-SIGN. IKKε is mostly known for its role in RIG-I-like receptor-mediated antiviral immunity via IRF3 and IRF7 phosphorylation to induce type I IFN production[45]. We have previously shown that IKKε recruitment to DC-SIGN in response to fucose binding leads to Bcl3-dependent priming of DCs for T_H2 polarization[20]. Here we demonstrated that IKKε also induced ISGF3-dependent T_{FH} differentiation in response to fucose-based PAMPs. The induction of both T_{FH} and T_H2 differentiation is required for production of high-affinity, class-switched antibodies and protective long-term humoural immunity[1,2]. IL-21 acts as a switch factor for human IgG1 and IgG3 (ref. 46). We show here that fucose priming of DCs induces differentiation of T_{FH} cells that functionally instruct B cells *in vitro* to induce IgG production. Sequential switching of IgG cells to IgE is essential for the development of long-lived high-affinity IgE^+ plasma cells, for which T_H2-produced IL-4 acts as a switch factor[4–6,8]. Thus, our results suggest that DC-SIGN-mediated DC priming via IKKε can provide the signals for establishing long-term humoural immunity via induction of both T_{FH} and T_H2.

The role of IKKε in T_{FH} differentiation revealed another level of crosstalk between innate signalling pathways. Here we demonstrated that crosstalk between IFNR and DC-SIGN signalling via IKKε is a prerequisite for T_{FH} differentiation. IKKε induced STAT1 phosphorylation at Ser708 that favours the formation of ISGF3 complexes by interfering with STAT1 homodimerization, hence increasing IL-27 production and inducing T_{FH} generation. IKKε phosphorylation of STAT2 in addition to STAT1 might further influence ISGF3 assembly[29]. However, although IKKε-mediated serine phosphorylation of STAT1 precedes TLR4-induced IFNR signalling, IFNR signalling is required for STAT1 phosphorylation at Tyr701 to induce heterodimerization between STAT1 and STAT2 (for ISGF3 formation) and STAT1 homodimerization[30,31]. A previous report suggested that phosphorylation of Ser708 and Tyr701 are mutually exclusive when Tyr701 phosphorylation preceded Ser708 phosphorylation[47]; however, during costimulatory TLR4-DC-SIGN triggering, Ser708 phosphorylation exhibits faster kinetics than Tyr701 phosphorylation. Thus, *de novo* type I IFN synthesis induced by TLR signalling intersects with IKKε signalling to generate ISGF3 complexes. IFNR signalling alone cannot replace TLR signalling as MK2 activation is required for LSP1 phosphorylation and subsequent IKKε activation after DC-SIGN triggering.

ISGF3 mediates its actions via ISRE sites and recruits RNAP2 to its target promoters, most likely via STAT2 within the ISGF3 complex[41]. Besides favouring ISGF3 formation via Ser708 phosphorylation, we also observed simultaneous Ser727 phosphorylation in an IKKε-dependent manner. Although Ser727 was not identified as a target for IKKε in mouse STAT1 (refs 28,29), we now show that IKKε is involved in Ser727 phosphorylation of human STAT1. Ser727 is present within the transactivation domain of STAT1, enhancing its transactivational activity[33]. Our results demonstrate that ISGF3 formation is not only dependent on DC-SIGN triggering but also implicate that ISGF3 activity is further boosted by IKKε-dependent DC-SIGN signalling.

Type I IFN signalling in DCs has previously been reported to be involved in the development of T_{FH} cells in mice via the induction of IL-6 (ref. 35). Our data show that in human DCs, IL-6 is a prerequisite but not sufficient for T_{FH} induction, while its induction via TLR4 is independent of IFNR triggering. Here we show that IFNR- and IKKε-dependent ISGF3 formation induces IL-27, which is the decisive cytokine for T_{FH} induction. The *IL-27* promoter contains a ISRE binding site that can be sequentially bound by IRF1 or IRF3 and ISGF3 (ref. 37). We observed initial transcriptional activation of *IL-27* due to TLR signalling before DC-SIGN-IKKε-induced ISGF3 activation, which was strongly accelerated once ISGF3 was bound and enhanced RNAP2 recruitment.

IL-27 most likely acts in concert with other factors at the time of DC priming to induce T_{FH} differentiation, such as IL-21 and IL-6 (refs 12,16). IL-27R deficiency also impairs T_{FH} induction[36]. IL-27, IL-21 and IL-6 all have in common that they induce STAT3 activation, implying that STAT3-mediated signalling routes in T cells are important for T_{FH} development[48]. Our data suggest a model in which threshold levels of IL-27 and IL-6 expression by DCs are required for efficient T_{FH} differentiation. LPS-stimulated DCs produced substantial IL-6 (ref. 20), but low amounts of IL-27, that was insufficient for T_{FH} differentiation. However, fucose-specific DC-SIGN signalling combined with LPS stimulation decreased IL-6 while enhancing IL-27 secretion, consequently inducing T_{FH} development. Parasites such as *S. mansoni* carry or secrete TLR3 and TLR4 ligands[39,40], underlining the physiological relevance of IFNR- and DC-SIGN-driven IL-27 expression during parasite infections.

Thus, we have identified the molecular mechanisms behind fucose-specific triggering of DC-SIGN signalling that leads to increased IL-27 expression by LPS-stimulated DCs, which triggers a threshold for differentiation of T_{FH} cells, which in turn induces specific IgG production by B cells. Unravelling the molecular requirements underlying DC-SIGN-mediated T_{FH} development will help to identify potentials targets for effective vaccination strategies as well as treatment of immunological diseases that are characterized by insufficient or excessive antibody responses.

Methods

Stimulation and RNA interference of DCs. Peripheral blood mononuclear cells were isolated from buffy coats of healthy volunteer blood donors (Sanquin) by a Lymphoprep (Axis-Shield) gradient step, and monocytes were subsequently isolated by a Percoll (Amersham) gradient step. Monocytes were differentiated into iDCs in the presence of 500 U ml^{-1} IL-4 and 800 U ml^{-1} granulocyte-macrophage colony-stimulating factor (GM-CSF) (both Invitrogen), and used at day 6 or 7. This study was approved by the Medical Ethics Review Committee of the Academic Medical Center (AMC).

DCs were stimulated with 10 ng ml^{-1} *Salmonella typhosa* LPS (Sigma), 10 μg ml^{-1} Pam3CSK4, 10 μg ml^{-1} poly(I:C) (both from Invivogen), 10 μg ml^{-1} Lewis X-PAA (Lectinity), 40 μg ml^{-1} SEA prepared from *S. mansoni* eggs (provided by F. Lewis, Biomedical Research Institute, Rockville, MD) or 40 μg ml^{-1} SPs prepared from adult *F. hepatica* worms (provided by L. Tielens, Utrecht University, The Netherlands). SEAs and SPs were prepared by homogenization of eggs or worms in 10 volumes of Tris–HCl (pH 8.0) via four

times 30 s polytron pulses on ice, followed by four times 1 min sonication (21 kHz, with 7 μm amplitude) on ice. After centrifugation (10,000 *g* at 4 °C for 30 min), supernatants were filtered (0.45 μm) and stored at −80 °C (ref. 49).

DCs were preincubated for 2 h with the following blocking antibodies: 20 μg ml^{-1} anti-DC-SIGN (AZN-D1; generated 'in-house'[50]), 20 μg ml^{-1} anti-IFNα/βR2 (MMHAR-2; PBL Interferon Source) or 20 μg ml^{-1} mouse IgG2a isotype control antibodies (14-4724-85; eBioscience). DCs were transfected with 25 nM short interfering RNA (siRNA) using transfection reagents DF4 (Dharmacon) according to the manufacturer's instructions. SMARTpool siRNAs used were as follows: LSP1 (M-012640-00), IKKε (M-003723-02), Bcl3 (M-003874-02), IRF9 (M-020858-02), IRF7 (M-011810-02) and non-targeting siRNA (D-001206-13) as a control (Dharmacon). Silencing of expression was verified by real-time PCR and flow cytometry; cell survival was determined by flow cytometry via Annexin V-fluorescein isothiocyanate (FITC)/PI staining (eBioscience) (Supplementary Fig. 4).

T-cell differentiation and B-cell help assay. Naive CD4$^+$ T cells were isolated from buffy coats of healthy blood donors (Sanquin) with human CD4$^+$ T-cell isolation kit II (Miltenyi) by negative selection combined with depletion of CD45RO$^+$ memory T cells by staining with phycoerythrin (PE)-conjugated anti-CD45RO (80 μg ml^{-1}; R0843; Dako), coated on anti-PE beads (Miltenyi). B cells were isolated from buffy coats of healthy blood donors (Sanquin) with human B-cell isolation kit II (Miltenyi) by negative selection; 95% of isolated cells were CD19$^+$, whereas 16% were IgD$^+$CD27$^-$ naive cells as determined by staining with anti-CD19 (1:25; HIB19; eBioscience), anti-IgD (1:20; IA6-2; Pharmingen) and anti-CD27 (1:20; LT27; eBioscience). This study was approved by the Medical Ethics Review Committee of the AMC.

DCs were either silenced for indicated proteins or preincubated for 2 h with blocking DC-SIGN antibodies and activated for 48 h as indicated. IDCs were cultured in the presence of 1 μg ml^{-1} GM-CSF (Invitrogen). DCs were cocultured with naive CD4$^+$ T cells (20,000 T cells/5,000 DCs) in the presence of 10 pg ml^{-1} *Staphylococcus aureus* enterotoxin B (Sigma). An amount of 30 ng ml^{-1} rhIL-27, 40 ng ml^{-1} rhIL-6 (both R&D Systems) or 5 μg ml^{-1} neutralizing antibodies against IL-27 (AF2526; R&D Systems), IL-6 (AF-206-NA; R&D Systems) or normal goat IgG (AB-108-C; R&D Systems), as a control, were added at this point. After 5 days, cells were further cultured with 10 U ml^{-1} IL-2 (Chiron). Resting T cells were restimulated after 12–16 days with 100 ng ml^{-1} PMA (Sigma) and 1 μg ml^{-1} ionomycin (Sigma). For flow cytometry analysis of differentiated T_H cells, cells were first fixed at 24 or 48 h after restimulation in 3% *para*-formaldehyde for 10 min and permeabilized in 90% methanol at 4 °C for 16 h. Cells were stained with Alexa Fluor 647-conjugated anti-CXCR5 (1:20; 558113; BD Pharmingen), PerCP-Cy5.5-conjugated anti-PD-1 (1:20; 561273; BD Pharmingen), Alexa Fluor 488-conjugated anti-Foxp1 (1:20; IC45341G; R&D Systems) and anti-Bcl-6 (1:20; ab19011; Abcam), followed by incubation with PE-conjugated anti-rabbit (1:200; 711-116-152, Jackson ImmunoResearch). Analyses were performed on a FACS Canto (BD Biosciences). Supernatants of T cells were harvested 24 h after restimulation for IL-21 expression analysis by enzyme-linked immunosorbent assay (ELISA), while mRNA was isolated as described below for quantitative real-time PCR analysis of T_H markers. For flow cytometry analysis of intracellular IL-4 (T_H2 cells) and IFN-γ (T_H1 cells) expression, cells were restimulated for 6 h, the last 4 h in the presence of 10 μg ml^{-1} brefeldin A (Sigma). Cells were stained with APC-conjugated anti-IL-4 (1:25; MP4-25D2; BD Biosciences) and FITC-conjugated anti-IFN-γ (1:5; 25723.11; BD Biosciences). DCs primed with 10 ng ml^{-1} LPS plus 1,000 U ml^{-1} IFN-γ (U-CyTech) or 1 μM PGE_2 (Sigma) were used as positive controls for T_H1 and T_H2 differentiation, respectively.

For analysis of T-cell-dependent help to B cells for Ig production, resting differentiated T cells were cocultured with B cells (100,000 T cells/50,000 B cells) and restimulated with 1 μg ml^{-1} anti-CD3 (1XE; Sanquin) and 1 μg ml^{-1} anti-CD28 (15E8; Sanquin). B-cell survival was examined after 2 days by flow cytometry via Annexin V-FITC/PI staining (eBioscience). B cells were identified by staining with anti-CD19-APC (1:40; 345791; BD Biosciences). Supernatants were harvested after 7 days for analysis of B-cell IgM and IgG production by ELISA.

DC-SIGN binding. DC-SIGN binding of parasitic PAMPs was determined by DC-SIGN-Fc ELISA as described in ref. 50.

Cytokine and Ig production. DC, T- or B-cell culture supernatants were harvested, and IL-21 (88-7216-22; eBioscience), IL-27 (88-7278-22; eBioscience), IgM (88-50620-22; eBioscience) and total IgG (88-50550-22; eBioscience) were measured by ELISA.

Quantitative real-time PCR. mRNA isolation, cDNA synthesis and PCR amplification in the presence of SYBR green in an ABI 7500 Fast PCR detection system (Applied Biosystems) were performed as described in ref. 20. Specific primers were designed using Primer Express 2.0 (Applied Biosystems; Supplementary Table 1). The relative mRNA expression was obtained by setting N_t ($=2^{Ct(GAPDH)-Ct(target)}$) at 1 in TLR ligand-stimulated DCs or T cells primed with LPS- (RORγt) or LPS/LeX- (Bcl-6 and IL-21) stimulated DCs, within one experiment and for each donor.

STAT1 and ISGF3 DNA binding. Nuclear extracts of DCs were prepared using NucBuster protein extraction kit (Novagen). STAT1 GAS DNA binding was determined using TransAM STAT family kit (Active Motif). ISGF3 ISRE DNA binding was determined using Universal EZ-TFA Chemiluminescent Transcription Factor Assay (Millipore) in combination with 5′-biotin-labelled sense strand oligonucleotide 5′-CTCTGCAAGGGTCATCGGGGAAGCCTTTTCAAGGAAAC GAAAGTGAACTC-3′ and unlabelled antisense strand 5′-GAGTTCACTTTCG TTTCCTTGAAAAGGCTTCCCCGATGACCCTTGCAGAG-3′. Bound ISGF3 was detected with anti-STAT1 (1:500; 9172; Cell Signaling), anti-STAT2 (1:100; 4594; Cell Signaling) and anti-IRF9 (1:500; sc-496X; Santa Cruz Biotechnology), followed by incubation with horseradish peroxidase (HRP)-conjugated anti-rabbit Ig (1:500; in kit) and chemiluminescence detection with a Synergy HT reader (Biotek).

STAT1 phosphorylation. Cells were first fixed in 3% *para*-formaldehyde for 10 min and permeabilized in 90% methanol at 4 °C for 30 min. Primary antibody incubation with anti-STAT1 p-S708 (ref. 47; 1:200; provided by M. Gale, Jr, University of Washington School of Medicine, Seattle, WA) and anti-STAT1 p-S727 (1:100; 9177; Cell Signaling) was followed by incubation with PE-conjugated anti-rabbit (1:200; 711-116-152, Jackson ImmunoResearch). STAT1 phosphorylation at Tyr701 was determined by direct labelling with Alexa Fluor 488-conjugated anti-STAT1 p-Y701 (1:1.67; 612596; BD Biosciences). Phosphorylation was analysed on a FACS Calibur (BD Biosciences). STAT1 phosphorylation was further determined in whole-cell extracts that were prepared using RIPA buffer (Cell Signaling). Proteins were resolved by SDS–polyacrylamide gel electrophoresis and detected by immunoblotting with anti-STAT1 p-S708 (1:250), anti-STAT1 p-S727 (1:1,000), anti-STAT1 p-Y701 (1:50) and anti-total STAT1 (1:1,000; 9172; Cell Signaling), followed by incubation with HRP-conjugated secondary antibody (1:2,500; 21230; Pierce) and ECL detection (Pierce).

ChIP assay. ChIP and re-ChIP assays were performed using ChIP-IT Express Enzymatic Shearing and ChIP-IT Express HT kits (both from Active Motif). Briefly, cells were fixed with 1% (vol/vol) *para*-formaldehyde, nuclei were isolated and chromatin DNA fragmented by enzymatic shearing (10 min, 37 °C). Protein–DNA complexes were immunoprecipitated using 4 μg anti-STAT1 (9172; Cell Signaling), anti-IRF9 (sc-496X; Santa Cruz), anti-RNAP2 (39097; Active Motif) or negative control IgG (sc-2027; Santa Cruz), and protein G-coated magnetic beads. For re-ChIP analyses, STAT1–DNA complexes were eluted from beads and desalted using the re-ChIP-IT kit (Active Motif). A second round of immunoprecipitation was performed with 4 μg anti-IRF9 or anti-STAT2 (39060; Active Motif). DNA was purified after reversal of crosslinks. Real-time PCR was performed with a primer set spanning both ISRE and TATA box of the *IL-27* promoter (Supplementary Table 1). Negative Control Primer Set 1 (Active Motif) was used as a negative control. To normalize for DNA input, a sample for each condition was taken along which had not undergone immunoprecipitation ('input DNA'); results are expressed as % input DNA.

Nuclear run-on assay. Nuclei were isolated from DCs as described previously[51]. Transcription was allowed to proceed in the presence of 1 mM 16-biotin-UTP (Roche) at 26 °C for 20 min. Newly synthesized biotin-containing RNA was isolated with mRNA capture kit (Roche). cDNA synthesis and PCR amplification were performed as described above, except that expression was normalized using β-actin. The relative mRNA expression was obtained by setting N_t at 1 in 1 h LPS-stimulated control-silenced DCs, within one experiment and for each donor.

Statistical analysis. Statistical analyses were performed using the Student's *t*-test for paired observations. Statistical significance was set at $P<0.05$.

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Author contributions

S.I.G. designed and supervised all aspects of this study, executed and interpreted most experiments, and prepared the manuscript. T.M.K. performed T cell differentiation and B cell help assays, FACS analyses and prepared extracts. B.A.W. contributed to ChIP assays and designed primers. M.v.d.V. performed time course experiments. E.J.K. and I.v.D. prepared the parasitic extracts. L.E.M.V. and M.A.W.P. de Jong performed DC-SIGN-Fc ELISAs. T.B.H.G. supervised all aspects of this study and helped prepare the manuscript.

Additional information

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