Fms-like tyrosine kinase 3 ligand administration overcomes a genetically determined dendritic cell deficiency in NOD mice and protects against diabetes development

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Abstract
A dendritic cell (DC) imbalance with a marked deficiency in CD4^+CD8^-1 DC occurs in non-obese diabetic (NOD) mice, a model of human autoimmune diabetes mellitus. Using a NOD congenic mouse strain, we find that this CD4^+CD8^-1 DC deficiency is associated with a gene segment on chromosome 4, which also encompasses non-MHC diabetes susceptibility loci. Treatment of NOD mice with fms-like tyrosine kinase 3 ligand (FL) enhances the level of CD4^+CD8^-1 DC, temporarily reversing the DC subtype imbalance. At the same time, fms-like tyrosine kinase 3 ligand treatment blocks early stages of the diabetogenic process and with appropriately timed administration can completely prevent diabetes development. This points to a possible clinical use of FL to prevent autoimmune disease.

The non-obese diabetic (NOD) mouse serves as a model of human autoimmune insulin-dependent diabetes mellitus, or type I diabetes. In these mice, as in the human disease, a gradually developing autoimmune response results in the destruction, by self-reactive T cells, of insulin-producing beta cells in the pancreatic islets (1, 2). In these mice, as in the human disease, predisposition to the autoimmune response is determined by multiple genetic factors (3, 4). The initiating events are unclear, but it seems likely that dendritic cells (DC) would be involved in the earlier stages of an autoimmune response. Indeed, DC, along with macrophages, are the first cells to infiltrate pancreatic islets at the onset of insulitis (5–9). Furthermore, NOD mice have been reported to have defects in DC development (10, 11).

Current models suggest that DC are crucial in maintaining the balance between tolerance and immunity (12–16). Thymic DC, by deletion of developing self-reactive T cells, are involved in central tolerance. Peripheral DC, in their immature or quiescent state, are considered to induce tolerance in self-reactive T cells which escape central tolerance, by T-cell deletion, by anergy or by production of regulatory T cells. On activation or maturation by microbial or inflammatory stimuli, the same DC can initiate immunity. This system sometimes malfunctions, activating self-reactive T cells and leading to autoimmune disease. However, it is not clear whether the distinction in function of quiescent versus activated DC applies to all of the multiple subtypes that are found amongst human and mouse DC populations. The CD8^+CD11c^+ conventional DC (CD8+ cDC) in the mouse may be crucially balanced in this respect. These DC have a constitutive ability to take up exogenous antigens and process them for ‘cross-presentation’ on MHC class I, and thereby activate CD8 T cells (17, 18). CD8^+ cDC are capable of driving CD8 T cells to the effector cytotoxic T-cell stage (19, 20). However, in the quiescent steady-state, CD8^+ cDC may be especially tolerogenic. They are the major DC subtype in the mouse thymus (21), and, possibly only because of this location, they have a role in central tolerance. CD8^+ cDC isolated from steady-state mouse peripheral lymphoid tissues produce a very restricted T-cell response in culture compared with CD8^- cDC (22–24). CD8^- cDC inhibit delayed hypersensitivity responses (25). Most pertinent to our present study, in a model in which ovalbumin is expressed by beta cells, CD8^- cDC in the lymph nodes draining the pancreas have been shown to present this model self-antigen to ovalbumin-specific CD8 T cells and induce peripheral tolerance (26, 27).

In view of these considerations, we analysed the DC subtype levels in diabetes-susceptible NOD mice. We found

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a selective deficiency in CD8+ cDC which mapped to a NOD mouse diabetes susceptibility locus on chromosome 4 (Chr4). Since we had previously shown that treatment of mice with the ligand for the receptor fms-like tyrosine kinase 3 (FL) not only elevates DC numbers overall (28) but selectively enhances both the CD8+ and pDC subtypes (29), we treated NOD mice with FL. This not only corrected the DC deficiency but also reduced, and in some conditions totally prevented, the development of diabetes.

Methods

Mice
All mice were produced at the Walter and Eliza Hall Institute (WEHI) animal breeding facility under specific pathogen-free conditions. The NOD/Lt females used typically have a cumulative diabetes incidence of 70–80% by 300 days in our facility. The control NOR/Lt females (30) and C57BL/6j females do not develop diabetes. To generate the NOD congenic strains described in this study, C57BL/6 females were crossed with NOD males. (NOD × B6)F1 females were then backcrossed to NOD males to generate backcross 1 generation. Ten subsequent backcrosses were then performed using the appropriate backcross progeny based on genotyping results and using NOD males or females to ensure that the Y chromosome and mitochondrial genome were NOD-derived. Genotyping results were based on DNA samples extracted from tail biopsies by standard methods and typed with polymorphic markers for Chr4, as well as a ~10 cM genome-wide scan including markers flanking previously described diabetes susceptibility loci (3). All marker positions and approximate centimorgan distances from the top of Chr4 were obtained from the Mouse Genome Database (www.informatics.jax.org).

FL treatment
Mice were injected subcutaneously once a day for 10 successive days with 10 μg FL (either human or mouse) in 0.1 ml PBS containing mouse serum albumin (MSA) 1 μg ml⁻¹, as described previously (28, 29). Control mice were injected with the PBS-MSA carrier solution only.

Diabetes assessment
Once a week from 80 days of age, the urine of mice was tested for glucose using BM-Test Glycemic® strips. Mice that were positive were then checked for blood glucose levels in a retro-orbital venous blood sample. Mice were considered diabetic if blood glucose was above 11 mM on two successive days. Diabetic mice were then killed and the pancreas removed for histological assessment.

FL
The recombinant mouse FL (mFL) was produced by a transfected CHO line provided by N. Nicola (WEHI) and was purified in our laboratory by affinity chromatography. The recombinant human FL (hFL), produced in a mammalian cell line, was provided by Searle (St. Louis, MO, USA). The functional effects and dose responses of both preparations on mouse DC populations have been presented previously (29).

DC isolation, enrichment and analysis
Full details of the procedures for DC isolation and flow-cytometric analyses have been presented previously (21, 31). To extract all DC, spleens or thymi were chopped and subjected to a mild collagenase digestion at room temperature and then treated with EDTA. This procedure does not activate DC. The lightest density cells were then selected by centrifugation in a Nycodenz medium. Non-DC lineages were removed by coating cells with mAb against CD3, Thy-1, CD19, GR-1 and erythrocytes, then depleting coated cells with anti-Ig magnetic beads. This procedure caused no loss of pDC. This DC-enriched preparation was then counted and labeled with up to four fluorochrome-conjugated mAb, together with propidium iodide. It was analyzed (and occasionally sorted) on a FACStar Plus instrument (Becton Dickinson, San Jose, CA, USA) using FL1 for FITC, FL2 for PE, FL3 for Cy5 and FL4 for Alexa 594, with the FL5 channel set to exclude propidium iodide-stained dead cells and autofluorescent cells. The primary division of DC was into pDC (CD11c⁺ CD45RA⁻) and cDC (CD11c⁺ CD45RA⁺). The cDC were sub-divided by additional staining into CD4⁻8⁻, CD4⁺8⁻ and CD4⁺8⁺ subtypes.

IL-12 production by DC
The assay procedures were as described in detail previously (32, 33). Purified sorted DC were cultured at 0.5 × 10⁶ cells ml⁻¹ in modified RPMI 1640 medium containing either fixed Staphylococcus aureus or the Toll-like receptor 9 agonist CpG 1668 (34) and an optimal mix of cytokines (granulocyte macrophage colony-stimulating factor, IFNγ and IL-4). The production of IL-12 p70 was determined 18 h later by an ELISA assay of the supernatants, using the mAb R29A5 as the capture antibody and C17.8 mAb as the detection antibody; note that the commonly used detection mAb R15D9 does not react with NOD IL-12 p70 and so gives a false-negative reading (H. Hochrein and M. O’Keeffe, unpublished results).

Results

DC subset levels in NOD mice
To determine if DC number or subtype balance was abnormal in NOD mice, the splenic DC populations were analyzed and compared with those of the diabetes-resistant but MHC-matched and genetically similar NOR strain (30), as well as with C57BL/6 mice. NOD differed from NOR in splenic DC number and balance, both at 55 days of age (Fig. 1, Table 1) and at 110 days (just before diabetes onset). At 110 days, proportions were similar to those found at 55 days but absolute DC levels were 30% lower in both strains (data not shown). NOD mice had a slightly reduced level of DC overall compared with NOR, a moderate reduction in CD4⁺8⁻ DC, but a more striking absolute and relative reduction in the CD8⁺ cDC subtype (P < 0.001). The major CD4⁺8⁻ splenic DC subtype was present at normal levels. This is in line with Prasad and Goodnow (10) who reported over-representation of CD8⁻ DC in NOD mice and Vasquez et al. (35) who reported a deficiency in CD8⁺ cDC. In contrast, we found no alteration in DC levels in the thymus, where CD4⁺8⁻ cDC are the major...
The DC in the NOD spleen presented similar levels of MHC II, co-stimulator molecules and a range of other markers to DC in NOR spleen (data not shown), which differs from the report of Prasad and Goodnow (10). The C57BL/6 mice were generally similar to the NOR controls in relative DC subset distribution, the only differences noted being lower surface expression of CD11c compared with both NOD and NOR, and a higher number of DC overall (Table 1). The relative deficiency in peripheral CD8+ cDC in NOD mice was apparent regardless of which strain was used for comparison.

Bioactive IL-12 production by CD8+ DC of NOD mice

To check if the CD8+ cDC that remained in NOD mice were normal in function, we tested their capacity to produce IL-12. CD8+ cDC normally have a much greater capacity to produce bioactive IL-12 p70 than other DC subtypes (33) and accordingly tend to induce Th1 responses (36, 37). Purified and sorted CD8+ cDC from NOD mouse spleen were cultured using stimuli previously shown to induce maximal IL-12 production (32). The NOD CD8+ cDC were able to produce substantial amounts of IL-12 p40 and of the bioactive IL-12 p70, although only about half that produced by CD8+ cDC from C57BL/6 mice (Fig. 2). It is not clear whether this partial reduction in IL-12 production capacity would have immunological consequences in vivo.

Genetic factors underlying the DC abnormality in NOD mice

To determine whether the genetic variation which contributes to the DC deficiencies in NOD mice also predisposes to diabetes onset, we used congenic mouse strains. We recently generated a NOD congenic mouse strain with a C57BL/6-derived interval on Chr4 (termed NOD.B6-Chr4). This interval is located distal to D4Mit31 (~51.3 cM) and encompasses all distal markers up to and including D4Mit256 (~82.7 cM). NOD.B6-Chr4 congenic female mice (n = 53) were monitored for diabetes and demonstrated a marked decrease (from 72% to 32% by 300 days of age, P > 0.001) in the incidence of diabetes compared with NOD female mice (n = 56). This result confirmed previous observations that one or more diabetes susceptibility loci mapped to this region (38, 39). We therefore compared the splenic DC subtype levels and balance in the NOD.B6-Chr4 congenic mice with those of NOD, NOR and C57BL/6.

Substitution of the C57BL/6-derived interval on Chr4 increased the level of CD8+ cDC in the spleen (P > 0.001) to the level in the NOR mice (Table 1, Fig. 3). The absolute level of DC overall did not rise and so remained below that of C57BL/6 mice. There was some increase in the levels of pDC and CD4-8+ DC, but these did not reach statistical significance. The overall result was that the relative levels of CD8+ cDC and the normal DC subtype balance was restored (Fig. 3). No changes in the levels of T cells, B cells or other haemopoietic cells were observed. There was no change in thymic DC (data not shown). The deficiency in peripheral CD8+ cDC is therefore determined by genes in this large interval of Chr4 of NOD mice, which includes known diabetes susceptibility loci.

Effect of FL administration on DC in NOD mice

In previous studies, we showed that administration of FL to C57BL/6 mice not only elevates DC levels overall (28) but selectively elevated the CD8+ cDC and the pDC subtypes...
This was especially apparent with mFL, although the overall elevation in DC levels was less than with hFL. Following FL treatment, levels of MHC II and co-stimulator molecules were characteristic of quiescent rather than activated DC. DC levels declined back to baseline 1 week after treatment. Since the CD8+ cDC subtype deficient in NOD mice was increased by FL in C57BL/6 mice, we asked if FL treatment could rectify the NOD DC imbalance.

NOD mice were treated with 10\( \mu \)g of either hFL or mFL per day for 10 days; then the DC levels and sub-population balance in the spleens were determined on day 11. Both treatments caused an increase in overall DC levels. The level of CD8+ cDC and pDC was markedly elevated, which became the dominant DC subtypes (Fig. 1, Table 1). The CD4\(^+\)/CD25\(^+\) and CD4+8\(^+\) cDC subsets increased but to a smaller extent than for CD8+ cDC.

Reduction in NOD mouse diabetes incidence following hFL treatment

Since FL treatment transiently corrected the DC imbalance in NOD mice, and for ~5 days even made CD8+ cDC the dominant subtype, we asked whether such treatment would reduce or delay diabetes incidence. Female NOD mice were treated at age 50 days with either hFL or mFL and the incidence of diabetes was serially monitored. The most striking effect was with hFL, which caused the greatest elevation in DC levels (29). The cumulative incidence of diabetes, which began ~100 days, was reduced from 68 to 14% (\( P > 0.001 \)) (Fig. 4).

Although FL treatment began at 50 days and the effect on DC levels would not have been evident beyond 70 days, there appeared to be a long-term effect to prevent diabetes development. In a parallel study, no NOR mice, whether FL-treated or untreated, became diabetic (data not shown).

Sections of the pancreas of hFL-treated NOD mice, untreated NOD mice and of control mice aged 195 days of age were examined histologically and assessed for mononuclear cell infiltration and destruction of pancreatic islets. The histology was in general accordance with the diabetes incidence results. The NOR control mice had a mean of 43 islets per seven spaced longitudinal sections and a mean of 57 islets per seven sections after hFL treatment; most islets showed mild peri-islet mononuclear cell infiltration, in contrast to C57BL/6 mice which showed no infiltration. Untreated NOD mice classed as diabetic had a mean of only 9 islets per seven sections, compared with those classed as non-diabetic which had 35 islets per seven sections; in both cases there was a high level of mononuclear cell infiltration in the remaining islets.

### Table 1. DC subtype levels in the spleen

<table>
<thead>
<tr>
<th>Strain</th>
<th>DC (x10^6) per spleen</th>
<th>Total pDC</th>
<th>Total cDC</th>
<th>CD4^+8^- cDC</th>
<th>CD4^+8^+ cDC</th>
<th>CD4^+8^- cDC</th>
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<tbody>
<tr>
<td>C57BL/6</td>
<td>0.61 ± 0.27</td>
<td>2.64 ± 0.40</td>
<td>0.53 ± 0.08</td>
<td>1.32 ± 0.20</td>
<td>0.50 ± 0.07</td>
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<tr>
<td>NOR</td>
<td>0.54 ± 0.10</td>
<td>1.78 ± 0.27</td>
<td>0.39 ± 0.06</td>
<td>0.93 ± 0.14</td>
<td>0.34 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>NOD</td>
<td>0.49 ± 0.14</td>
<td>1.53 ± 0.17</td>
<td>0.18 ± 0.02</td>
<td>1.09 ± 0.12</td>
<td>0.21 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>NOD, FL treated</td>
<td>12.74 ± 3.76</td>
<td>18.58 ± 2.23</td>
<td>11.75 ± 1.91</td>
<td>2.36 ± 0.48</td>
<td>3.92 ± 1.01</td>
<td></td>
</tr>
<tr>
<td>NOD.B6-Chr4</td>
<td>0.56 ± 0.13</td>
<td>1.71 ± 0.40</td>
<td>0.39 ± 0.09</td>
<td>0.94 ± 0.22</td>
<td>0.27 ± 0.06</td>
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</table>

The total level of various DC subtypes in the spleens of NOD mice, compared with NOR and C57BL/6 control mice and with congenic NOD.B6-Chr4 mice. The effect of FL treatment on the levels in NOD mice is also presented. Results were calculated from a total cell count in the DC-enriched preparation combined with the relative levels in analyses such as Figs 1 and 3. The mice were ~55 days of age. Results represent the means ± SD of five experiments, except for the FL treated which are the means ± range of two experiments. All assays were on a pool of at least three spleens.
islets and partial destruction of the beta cells. The hFL-treated NOD mice, which were non-diabetic (the majority), had a mean of 47 islets per seven sections, with the islets showing a moderate level of mononuclear cell infiltration but only a low level of beta-cell destruction.

Complete prevention of diabetes development with repeated doses of mFL

We have previously found that mFL was less effective than hFL in enhancing DC number (29). Our preliminary experiments suggested that, in contrast to hFL, a single administration of mFL only transiently inhibited diabetes development. Therefore, we explored the effects of different times of administration of mFL. When NOD mice were given 10 days of mFL treatment beginning at age 20 days, when the autoimmune process is just beginning, there was a small but not statistically significant reduction in diabetes incidence between 100 and 150 days of age, but no difference thereafter (Fig. 5). When mFL was administered beginning at 50 days of age, there was an apparent reduction in diabetes incidence between 120 and 200 days, but in contrast to the results with hFL, the effect was of marginal significance and eventually the mice became diabetic (Fig. 5). When mFL was administered at 100 days, when beta-cell destruction is beginning, there was no evidence of immediate protection from diabetes development, but the data suggested a reduction in late-onset diabetes incidence, from 250 to 370 days (Fig. 5).

This set of marginal results suggested the hypothesis that mFL treatment provided only a transient window of protection from the initiation of an autoimmune process that could begin at any time over the young adult life of NOD mice, cumulating in diabetes ~100 days later. If this was correct, then repeated administration of mFL, covering all the ‘windows,’ might block initiation of the autoimmune process entirely. To test this hypothesis, we combined in one group of NOD mice all the previous mFL treatments, by giving mFL for 10 days starting at 20 days, again at 50 days and again at 100 days of age. This repeated administration of mFL had the predicted effect of preventing diabetes development completely (P > 0.001) (Fig. 5).

Discussion

These results can be considered at two levels. One is the role of DC in the initiation, prevention and regulation of autoimmune responses, such as those that lead to type 1 diabetes. The second is the practical issue of an appropriate intervention to prevent autoimmune pathology in genetically susceptible individuals. Our results suggest that FL administration could delay or prevent the onset of type 1 diabetes and possibly other autoimmune diseases.

The general picture of type 1 diabetes is of a progressively evolving autoimmune process with the final destruction of the beta cells of the pancreas being a long-term outcome of earlier initiation events (1, 2). Since some self-reactive cells are present in all individuals, the initiation of autoimmunity may be due to a failure to regulate self-reactivity rather than an exogenous initiation event. In the case of NOD mice, there is clearly a strong stochastic element, since not all of these genetically identical mice eventually develop diabetes. Our results with administration of mFL to NOD mice of various ages suggest that the early events leading to beta-cell destruction some 100 days later may occur at any time between 20 and 120 days of age. Thus, interventions aiming for complete prevention of disease must have a continuing effect over this long vulnerable period.

Our results suggest that DC play a critical role in the prevention of these early events, which in NOD mice eventually lead to the autoimmune destruction of the beta cells. If all our data are combined, they point to the peripheral CD8+ cDC as being the most likely candidate for this protective role. We present three arguments for this. First, as we and others (10, 35) have found, CD8+ cDC are deficient in NOD mice, the relative deficit being greater than for pDC or CD4+ b2-DC. Secondly, this relative deficit of CD8+ cDC (and not any deficit in total DC number or in other haemopoietic cells) is genetically determined by genes in an interval of Chr4 which includes a non-MHC diabetes susceptibility locus. Thirdly, elevating the relative level of CD8+ cDC by administering FL, to the point where they become the major cDC subtype, results in a striking suppression of diabetes development in NOD mice. While the manuscript was under review, a study by Chilton et al. (40) was published showing that hFL administration partially prevented diabetes in NOD mice, with data very close to our own.

In our study, appropriate FL administration provided greater protection against diabetes than having the protective gene on Chr4, perhaps because it resulted in abnormally high levels of CD8+ cDC. While this represents strong circumstantial evidence, it does not constitute a proof that CD8+ cDC protect against the autoimmune response. Our continuing genetic studies offer the best chance of determining whether CD8+ cDC are the crucial cells because DC transfer studies are limited and any interpretation is confused by the short life span and poor re-seeding capacity of isolated, purified DC.

There are several levels at which murine CD8+ cDC could regulate autoimmunity. We assume they would be tolerogenic.
in their quiescent state, rather than when fully activated when they would initiate immunity. They could function via central tolerance, with higher levels of thymic CD8+ cDC serving to enhance the elimination of developing self-reactive T cells. However, our genetic studies only pointed to differences in peripheral, not thymic, DC. Although FL treatment would elevate thymic as well as peripheral DC, most of the developing T-cell repertoire should be outside the thymus by 50 days of age, when FL was still effective. A more likely scenario would be that in normal mice, peripheral steady-state CD8+ cDC continuously suppress the responses of self-reactive T cells that have escaped thymic deletion. This could be by a direct activation-dependent deletion of the self-reactive T cells or by promoting the generation of regulatory T cells. Since the life span of T cells is longer than that of DC, generation of a regulatory T-cell population could explain the long-term effects of a transient increase in DC levels in the NOD mice. In either case, a genetically determined deficit of CD8+ cDC would predispose to autoimmunity and the increase on FL treatment would be protective.

Other possible roles for CD8+ cDC should also be considered. They might regulate the type of T-cell response obtained, deflecting it away from a pathological Th1 response, as proposed in a recent study in which FL-induced DC were transferred into NOD mice, with a resultant protection against diabetes (41). However, this seems an unlikely explanation for our results, since CD8+ cDC are major producers of IL-12 p70 which promotes rather than prevents Th1 responses (30, 33) and which may accelerate diabetes even in the absence of IFNγ (42). This applies to the CD8+ cDC from normal mice (32), from FL-treated mice (29) and as we now show from NOD mice. Another possible role for CD8+ DC is in maintaining T-cell homeostasis. A recent study (43) has suggested that autoimmunity may be a consequence of a lymphopenic state and that NOD mice are mildly lymphopenic. If CD8+ DC are specialised for this role, increasing their number might elevate the T-cell level. A final, if unlikely, possibility is that CD8+ cDC function not to inhibit the autoimmune response, but to promote immunity to some unknown viral infection, either latent within the beta cell or which cross-reacts with beta-cell components. Enhancing CD8+ cDC, which efficiently present viral antigens to T cells (44), would prevent infection and so indirectly protect against diabetes development. Enhancing pDC by FL treatment would also contribute to resistance to viral infections.

There was a surprising difference between the long-term protection against diabetes development afforded by a single treatment with hFL in our study and that of Chilton et al. (40), and the short-term effects and the need for multiple treatments we found necessary using mFL. The requirement for multiple treatments with mFL is in line with the short life span of CD8+ DC (45) and suggests a direct and continuous involvement of

**Fig. 5.** Cumulative diabetes incidence in female NOD mice treated at various ages with mFL. The control group was injected with carrier medium alone. The upper graphs (n = 16 per group) present the results from mice given a single 10-day treatment beginning at 20 days, at 50 days or at 100 days of age. The lower graph (n = 18 per group) are from mice given three successive 10-day treatments beginning at 20 days, then at 50 days and then at 100 days of age.
this DC subtype. In contrast, the results with hFL point to the involvement of a cell with a longer life span, such as pDC or a regulatory T cell. Determining the exact mechanism behind the protective effect of FL treatment will obviously require considerable further experimentation, and this is underway.

Regardless of whether CD8+ cDC levels are crucial variables, the protective effect of FL treatment suggests a therapeutic potential. Our experiments raise the possibility that FL treatment, either alone or in conjunction with other tolerogenic procedures (46) could prevent diabetes developing in genetically susceptible humans. It has been demonstrated that hFL elevates DC levels in humans, as it does in mice (47). Although CD8 is not expressed on human DC and the equivalent DC subtype has not yet been identified in human spleen or lymph nodes, it seems likely that an equivalent would exist. Our studies have shown a close parallel between the DC in mouse and human blood (48), and it is probable that similar subtypes exist. Our experiments have shown a close parallel between the DC in mouse and human blood (48), and it is probable that similar subtypes exist.

In our studies, we have shown that DC subtypes have been identified in human spleen or lymph nodes, it seems likely that an equivalent DC subtype has not yet been identified in human spleen or lymph nodes, it seems likely that an equivalent would exist. Our studies have shown a close parallel between the DC in mouse and human blood (48), and it is probable that similar subtypes exist. Our experiments have shown a close parallel between the DC in mouse and human blood (48), and it is probable that similar subtypes exist.

Although we have insufficient data to suggest a clinical protocol for FL use, some important parameters are evident. A pre-requisite would be the ability to select children who have a high probability of developing the disease. This is already feasible (51). If our data on mFL injected into mice are a guide to hFL administered to human, there could be a requirement for prolonged FL presence to prevent the disease. Rather than rely on repeated administration, it may be necessary to modify FL to prolong its half-life in serum (52).

If FL was effective in protecting NOD mice from diabetes because it increased the level of quiescent, tolerogenic DC, activation of these DC, by microbial stimuli or inflammation, might be predicted to reverse the protective effect and even to promote the autoimmune process. Extensive pre-clinical testing of the effects of FL treatment, combined with simultaneous activation of DC using a range of stimuli, will be needed before further consideration of its use for treating human autoimmune disease.

Acknowledgements

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Chr4</td>
<td>chromosome 4</td>
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<tr>
<td>cDC</td>
<td>conventional DC</td>
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<tr>
<td>DC</td>
<td>dendritic cells</td>
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<tr>
<td>FL</td>
<td>ligand for Fms-like tyrosine kinase 3</td>
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<tr>
<td>hFL</td>
<td>human FL</td>
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</table>

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