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*J Immunol* 2008; 181:1753-1759; doi: 10.4049/jimmunol.181.3.1753
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Influence of a Non-NK Complex Region of Chromosome 6 on CD4⁺ Invariant NK T Cell Homeostasis

David Vallois,* Marie-Claude Gagnerault,* Philip Avner,† Ute C. Rogner,† Christian Boitard,* Kamel Benlagha,* André Herbelin,‡ and Françoise Lepault2*§¶

The number and function of immunoregulatory invariant NKT (iNKT) cells are genetically controlled. A defect of iNKT cell ontogeny and function has been implicated as one causal factor of NOD mouse susceptibility to type 1 diabetes. Other factors of diabetes susceptibility, such as a decrease of regulatory T cell function or an increase in TLR1 expression, are corrected in diabetes-resistant Idd6 NOD.C3H 6.VIII congenic mice. Thus, we surmised that the iNKT cell defects found in NOD mice may also be rescued in congenic mice. Unexpectedly, we found, in both the thymus and the periphery, a 50% reduction in iNKT cell number in NOD.C3H 6.VIII mice as compared with NOD mice. This reduction only affected CD4⁺ iNKT cells, and left the double negative iNKT cells unchanged. In parallel, the production of IL-4 and IFN-γ following α-GalCer stimulation was proportionally reduced. Using three subcongenic strains, we have narrowed down the region controlling iNKT development within Idd6 (5.8 Mb) to Idd6.2 region (2.5 Mb). Idd6 region had no effect on NK cell number and in vivo cytotoxic activity. These results indicate that the role of iNKT cells in diabetes development is equplico and more complex than initially considered. In addition, they bring strong evidence that the regulation of CD4⁺ iNKT cell production is independent from that of DN iNKT cells, and involves genes of the Idd6 locus. The Journal of Immunology, 2008, 181: 1753–1759.

Invariant NK T cells, an unconventional T cell population that play important immunoregulatory roles, express an invariant Ag TCR α-chain (Vα14α18 in the mouse and Vα24β18 in human), together with several NK cell receptors (1). In mice, iNKT cells are composed of two subsets: the CD4⁺ population and the double negative (DN) or CD4⁻ iNKT cell subset, the latter arising in part from immature CD4⁺ iNKT cells (2, 3). iNKT cells are capable of rapidly producing large amounts of cytokines, notably IL-4 and IFN-γ, upon stimulation by glycolipid-type ligands presented by the MHC class I-like CD1d molecule. The first identified iNKT cell ligand, α-galactosylceramide (α-GalCer), was isolated from marine sponges and allowed the visualization of Vα14 NK T cells. More physiological ligands have been described, such as microbial lipids and the self-ligand iGb3 (1, 4).

The NOD mouse develops type 1 diabetes characterized by T cell dependent destruction of insulin-producing β-cells in the pancreas. Lack of tolerance to islet cell-related Ags has been ascribed to deficiencies in regulatory T cell subsets such as naturally arising CD4⁺CD25⁺ regulatory T cells, CD4⁺CD25⁻CD62L⁺ T cells (5, 6) and invariant NK T cells (iNKT cells) (7–9). NK cells were also reported to be deficient in NOD mice, and a role of NK cells in type 1 diabetes has been evoked (10–13).

NOD mice exhibit both a numerical and a functional defect of iNKT cells detectable as early as 3 wk of age when compared with normal strains (7). The protective role of iNKT cells is suggested by their capacity to inhibit disease transfer by diabetogenic T cells, and by the reduction of diabetes, prevalence in NOD mice transgenic for the Vα14Jα18 TCR chain (14) or overexpressing CD1d in pancreatic islets (15). Diabetes can also be prevented by vivo treatment with α-GalCer (16–19).

Interstrain differences suggest that the number and function of iNKT cells are genetically controlled. A genome-wide screen of a cross between NOD and C3HBL/6 mice identified two major loci controlling iNKT cell number: Nkt1 on distal chromosome 1, and Nkt2 on chromosome 2 that overlaps with the insulin-dependent diabetes susceptibility locus Idd13 (20, 21). iNKT cell abnormalities in NOD mice were clearly shown to be independent of Idd1, Idd3, Idd11, and Idd17/I018 regions (22, 23), but some controversy remains concerning Idd5 and Idd9 (22, 24). Other Idd loci, including Idd6 and Idd13, contribute to iNKT cell homeostasis (4, 24, 25). These data suggest that the iNKT cell defect in NOD mice might be a genetically determined component of their diabetes susceptibility. Reduction in iNKT cell number and function is not an invariant correlate of enhanced autoimmunity: lupus-prone mice exhibit an increased number and activity of iNKT cells (24). Taken together, these observations indicate that there is a complex genetic relationship between defects in iNKT cell development and autoimmune susceptibility.

Recently, we have undertaken a detailed phenotypic analysis of the diabetes-resistant NOD.C3H 6.VIII (6.VIII) congenic strain,
carrying C3H alleles at the 5.8 Mb Idd6 interval on distal chromosome 6 excluding the NK complex, and showed that regulatory CD4+CD25+ T cell population expressed an enhanced inhibitory activity in 6.VIII mice (26). However, CD4+CD25+ T cells may not be the only regulatory T cells involved in the protection seen in 6.VIII congenic mice. To evaluate the role of the immunoregulatory NK and iNKT cells in diabetes-resistant 6.VIII congenic non-autoimmune prone strains, and unexpectedly, we found that functional defects of iNKT cells in the NOD mouse compared with nondiabetes development, showed that this deficiency localizes to the Idd6.2 subinterval (27).

Materials and Methods

Mice

All mice were bred and housed in specific pathogen-free conditions and used at 6–15 wk of age. The NOD control strain (bred from congenic mouse littermates), the congenic strain NOD.C3H 6.VIII (6.VIII) and the three subcongenic strains 6.VIIIa, 6.VIIIb, and 6.VIIIc are as described (26, 27). NOD, NOD.β2M−/−, and C57BL/6.I-Ak7 mice were maintained by brother-sister mating. C3H/HeJ and C57BL/6 mice were purchased from Centre Janvier. The relevant Institutional Review Boards approved animal studies.

Diabetes prevalence at 30 wk of age in females is 85–90% for NOD mice, 45–55% for 6.VIII congenic mice, and 70–75% for all three subcongenic strains, and the severity of insulitis was equivalent in pancreas from NOD and 6.VIII mice (26, 27).

Reagents

A synthetic form of α-GalCer (KN-7000; Pharmaceutical Research Laboratory, Kirin Brewery) was used. α-GalCer-loaded CD1d tetramers labeled with allophycocyanin were prepared in house. Anti-CD4, anti-IL-4, anti-IFN-γ, anti-CD69, anti-CD45, anti-HSA, and DX5 (CD49b) were purchased from BD Pharmingen. Purification and FITC labeling of Abs against TCR-β, Vβ8, Vβ7, Vβ2, CD4, CD8, and CD16/CD32 (2.4G2) was conducted in our laboratory.

Cell preparation and flow cytometry analysis

Single-cell suspensions of splenocytes and thymocytes were prepared using standard techniques. Livers were perfused with 1% heparin in PBS, passed through a 42-μm cell strainer, and cells washed three times in PBS/5% FCS. Hepatic lymphocytes were then separated using a 35% Percoll solution. RBCs were lysed in an ammonium chloride buffer. Isolation of leukocytes from pancreas was performed as described (26). Spleen, thymus, and liver mononuclear cells, after surface staining, were analyzed using a FACSCalibur cytometer and CellQuest software (BD Biosciences). Dead cells were excluded from analysis using forward and side-scatter parameters. A minimum of 10^5 CD1d−/−α-GalCer tetramer-TCRβ+ cells were acquired in each run. The absolute number of iNKT cells was calculated from the percentage of the CD1d−/−α-GalCer tetramer positive cells and the total cell number in each organ.

NK cells were enumerated in spleen, bone marrow, and pancreas as DX5+ TCRβ− cells.

In vivo NK cell cytotoxicity assay

NK cell activity was studied in vivo according to a modification of the method described by Bix et al. (28) and Poultten et al. (12). In brief, bone marrow cells from NOD, 6.VIII, and NOD.β2M−/− mice were depleted of T cells by negative selection of cells labeled with biotinylated anti-CD4 and anti-CD8 Abs followed by streptavidin magnetic beads and sorted using a VarioMACS device (Miltenyi Biotec). Purity of harvested non-T cells was >99%. Cells were labeled at 10 × 10^6 cells/ml with 5 μM CFSE (Invitrogen), washed, and injected in the retro-orbital sinus of lethally irradiated (10 Gy) recipients. Three days later, spleen cells from all recipients were stained with anti-CD45 Ab and CD45+ CFSE+ cells were analyzed.

Statistics

Results are presented as mean ± SEM. Groups were compared by using Mann-Whitney test or one-way ANOVA followed by multiple comparisons of means with Tukey test.

Results

Idd6 locus does not influence NK cell number and cytotoxic activity

We analyzed the possible influence of the Idd6 region on the number and lytic function of NK cells. Cell suspensions were stained with DX5 and anti-TCR-β Abs. No significant differences were found in the frequencies and numbers of DX5+ TCRβ− cells between NOD and 6.VIII strains in spleen (1.91 ± 0.26 × 10^6 and 2.11 ± 0.12 × 10^6, respectively), bone marrow (4.4 ± 0.5 × 10^5 and 3.5 ± 0.1 × 10^5, respectively), and pancreas (4.5 ± 0.9 × 10^3 and 4.5 ± 1.6 × 10^3, respectively).

NK cell cytotoxic activity was studied in vivo according to a method described previously (28, 29) evaluating survival of control and MHC class I deficient (β2M−/−) cells in the spleen.
of lethally irradiated recipients (Fig. 1). After injection of NOD, β2M−/− cells, ~3 times fewer cells were recovered in both NOD and 6.VIII recipients than in mice injected with normal cells. In C57BL/6.1A87 recipients at least 10 times less donor cells survived. A similar cytotoxic activity was observed in NOD.scid recipients indicating that β2M−/− cell killing was T cell-independent. Survival of NOD and 6.VIII cells in both recipients was comparable. These data suggest that the killing defect of NK cells in NOD mice is not under the control of the Idd6 region.

Numerical and activation profiles of iNKT cells in Idd6 congenic mice

NOD mice present a numerical defect in iNKT cells compared with BALB/c and C57BL/6 control mice (7, 29), and protection against diabetes was achieved by increasing the number of endogenous iNKT cells (14). To test whether protection in the 6.VIII congenic strain was partly due to normalization of iNKT cell numbers and function, thymus, spleen, and liver iNKT cells were enumerated using a CD1d tetramer probe. iNKT cells were clearly identified in lymphocyte preparations as α-GalCer-loaded CD1d tetramer−/− expressing an intermediate level of TCR-β (Fig. 2A). As expected, iNKT cell frequencies and numbers in thymi, spleens, and livers of NOD mice were significantly reduced compared with C3H mouse organs. Remarkably, 6.VIII mice showed a significant decrease in the proportion and absolute number of iNKT cells as compared with NOD mice (Fig. 2B). In the thymi, spleens, and livers, the iNKT cell numbers of 6.VIII mice represented roughly 50% those of NOD mice, and 25–30% those of C3H mice. Conversely, congenic mice showed no modification of the frequencies and numbers of conventional CD4+ and CD8+ T cells (Fig. 2C).

We next determined how the reduction in iNKT cells in 6.VIII mice related to the CD4+ and DN iNKT cell subsets. In the thymus, liver, and spleen, the frequency of CD4+ cells among the iNKT cell population was 2 times greater in 6.VIII mice than in NOD mice, and 30–50% those of C3H mice. In addition, we have shown that introgression of the C3H mouse Idd6 region did not modify the number and activity of pathogenic T cells (26).

FIGURE 2. Distribution of iNKT cells in congenic 6.VIII, NOD, and C3H mice by flow cytometry. A. Cells were labeled with allophycocyanin-CD1d-α-GalCer tetramers and FITC-anti-TCRβ (thymus cells) or PE-anti-HSA (liver and spleen cells) Abs. Values represent the mean ± SEM of the frequency of iNKT cells in each gated region, from the number (n) of mice indicated in the histogram legend. B. Histograms show frequency and absolute number of iNKT cells in each organ. (p < 0.05; *, NOD vs 6.VIII; †, NOD vs C3H; ‡, 6.VIII vs C3H). C. Frequency of conventional CD4+ and CD8+ T cells in the spleen of NOD, 6.VIII and C3H mice (p > 0.05), after labeling with anti-CD4 and anti-CD8 Abs.
found in NOD mice, and this defect exclusively affected CD4
targets. 6.VIII vs C3H; ‡, NOD vs C3H). Representative FACS analysis of CD4
liver cells (Fig. 3, though significant, this difference was much less pronounced for
and 6.VIII mice was significantly lower than in C3H mice. Al-
FE
cells from NOD (Fig. 3,CD4
liver cells (Fig. 3). Interestingly, the proportion of CD69
expression. Cells were labeled as described in Materials and	methods. (A) and (B), The mean ± SEM of the absolute number of CD4+ and DN
(CD4-) cells from each organ is shown. C, CD4+/DN iNKT cell ratio. D, Frequency of iNKT cells expressing CD69. (p < 0.05; *, NOD vs 6.VIII; †, 6.VIII vs C3H; ‡, NOD vs C3H). Representative FACS analysis of CD4 and CD69 expression on gated TCR
†, 6.VIII vs C3H; ‡, NOD vs C3H). Representative FACS analysis of CD4 and CD69 expression on gated TCRβCD1d-α-GalCer tetramer+ liver cells from NOD (E) and 6.VIII (F) mice. The numbers of mice analyzed were as in Fig. 2.

NOD than in 6.VIII mice (Fig. 3C). We also analyzed the iNKT cell content of pancreas infiltrates of males and females NOD and 6.VIII mice aged 13–15 wk. A reduction in the iNKT cell proportion was also observed in 6.VIII mouse pancreas (1.3 and 0.7% of TCRβ+ cells in NOD and 6.VIII females, respectively; and 1.6 and 0.5% in NOD and 6.VIII males, respectively). The CD4+/DN ratio of pancreatic iNKT cells (2.3 and 0.8 in NOD and 6.VIII females, respectively; and 2.1 and 1.2 in NOD and 6.VIII males, respectively) was also decreased in 6.VIII mice, and similar to that of thymus and liver. Unlike Naumov et al. (17), who reported a decline in iNKT cell number exclusively in female islets, we found a decrease in the infiltrates of both males and females. This discrepancy is likely due to the fact that they quantified Vα14Jα18 transcripts rather than cells.

iNKT cells are memory/activated cells expressing high levels of CD69 in control mice. This was confirmed for C3H mice (Fig. 3D). Interestingly, the proportion of CD69+ iNKT cells in NOD and 6.VIII mice was significantly lower than in C3H mice. Although significant, this difference was much less pronounced for liver cells (Fig. 3, D–F).

Our data clearly establish that introgression of the C3H Idd6 region worsened the numerical defect of iNKT cell population found in NOD mice, and this defect exclusively affected CD4+ iNKT cells in the thymus and the peripheral organs.

**IFN-γ and IL-4 production in Idd6 congenic mice**

A hallmark of iNKT cells is their capacity to promptly release large amounts of both IFN-γ and IL-4 after in vivo treatment with α-GalCer (8, 21). To analyze iNKT cell cytokine production, 6.VIII congenic and NOD mice were injected i.v. with PBS or 2 µg of α-GalCer and bled 90 min later. Levels of IFN-γ and IL-4 in the serum of 6.VIII mice were, respectively, 46 and 38% of those found in NOD mice (Fig. 4).

Thus, the numerical defect of 6.VIII iNKT cells is associated with defective cytokine release upon α-GalCer activation. To determine whether this low release of cytokines simply reflected the reduction in cell numbers or was also due to functional defects of the cells, we assessed cytokine expression at the single-cell level 90 min after i.v. injection of α-GalCer or PBS in NOD, 6.VIII, C3H, and C57BL/6 mice. Fig. 5 shows the functional status of hepatic CD4+ and DN iNKT cells. Regardless of the iNKT cell subset tested, C57BL/6 and C3H/HjeJ iNKT cells had similar patterns of cytokine production. We therefore pooled results from both control strains, which were considered as nonautoimmune mice.

IFN-γ and IL-4 responses of CD4+ and DN iNKT cells were significantly decreased in mice of NOD background as compared with nonautoimmune mice. No differences were detected between NOD and 6.VIII mice (Fig. 5). Thus, the reduction of cytokine levels in the blood was a direct consequence of the numerical defect in iNKT cells. However, in both NOD and 6.VIII mice, the frequency of DN iNKT cells producing IL-4 was more severely reduced (2.3-fold) than that of CD4+ iNKT cells (1.4-fold). Mean fluorescence intensities were similar, suggesting that cytokine content in IFN-γ or IL-4 producers was likely equivalent in all mouse strains tested (data not shown).

Idd6 does not influence the Vβ repertoire of iNKT cells

To determine whether 6.VIII mouse iNKT cells showed the same restricted TCR Vβ-chain repertoire as control mice, we analyzed Vβ8, Vβ7, and Vβ2 distribution within thymus iNKT cells from 8-wk-old NOD, 6.VIII and C57BL/6 females. Wild-type and congenic mice of the NOD genetic background display the same Vβ repertoire as control mice, we analyzed

FIGURE 3. Influence of Idd6 locus on iNKT cell subset number and CD69 expression. Cells were labeled as described in Materials and Methods. A and B, The mean ± SEM of the absolute number of CD4+ and DN (CD4-) cells from each organ is shown. C, CD4+/DN iNKT cell ratio. D, Frequency of iNKT cells expressing CD69. (p < 0.05; *, NOD vs 6.VIII; †, 6.VIII vs C3H; ‡, NOD vs C3H). Representative FACS analysis of CD4 and CD69 expression on gated TCRβCD1d-α-GalCer tetramer+ liver cells from NOD (E) and 6.VIII (F) mice. The numbers of mice analyzed were as in Fig. 2.

**FIGURE 4.** In vivo cytokine responses of NOD and 6.VIII mice. Serum levels (mean ± SEM) of IFN-γ (A) and IL-4 (B) were measured by ELISA 2 h after an i.v. injection of 2 µg of α-GalCer per mouse. (p = 0.0171 for IFN-γ; p = 0.0017 for IL-4). n = number of mice analyzed.
6.VIIIb, and 6.VIIIc strains, that carry the subloci Idd6.1, Idd6.2, and Idd6.1 and 3, respectively (27). The three subcongenic strains are significantly protected from diabetes compared with the wild-type NOD strain, although each strain is slightly less protected than the 6.VIII strain (27). We analyzed the number and distribution of iNKT subsets in the thymus of 6.VIII, 6.VIIIa, 6.VIIIb, and 6.VIIIc mice. The 6.VIIIb mice shared with 6.VIII mice the characteristics of iNKT cells that distinguish them from NOD mice, that is a low number of total iNKT resulting from a reduced number of CD4\(^{+}\) iNKT cells (Fig. 7, A and C) and a low CD4\(^{+}\)/DN iNKT cell ratio (Fig. 7B). The distribution of iNKT cell subpopulations in 6.VIIIa and 6.VIIIc strains were similar to those of NOD mice. These results suggest a control of iNKT cell development by Idd6.2 locus.

Frequencies of CD69\(^{+}\) iNKT cells were identical in all subcongenic mice, and similar to those found in NOD and 6.VIII mice (data not shown). This confirms the incomplete maturation of iNKT cells on the NOD background.

**Discussion**

In the current study, we analyzed iNKT cells in the Idd6 congenic strain NOD.C3H 6.VIII. We show that fewer iNKT cells reside in diabetes resistant 6.VIII mice than in wild-type NOD mice. This reduction affected only the CD4\(^{+}\) subset. This numerical defect is accompanied by a decrease in the production of IL-4 and IFN-\(\gamma\). We provide the first evidence that genes within the Idd6.2 subregion specifically influence CD4\(^{+}\) iNKT cell homeostasis. NK cell number and cytolytic activity were not modified in 6.VIII mice.

The finding that NOD mice show a severe deficiency in iNKT cell number and function (7) prompted the suggestion that iNKT cells play a protective role in diabetes development. However, despite a large body of information, the true physiological role of iNKT cells in diabetes development remains puzzling. Investigations in genetically modified NOD mice harboring either increased...
numbers of iNKT cells as compared with wild-type NOD mice, or no iNKT cells brought discrepant data. Transgenic mice for a Vα14-Jα18 TCR containing high levels of iNKT cells (14) were protected, and introgression of the C57BL/6 Nkt1 locus corrected the iNKT cell defects but did not alter the course of spontaneous diabetes (21). Diabetes incidence was found either increased (16, 30) or unchanged (19, 31) in CD1d-deficient NOD mice, and no effect was observed in Jα18-deficient mice (Ref. 31 and A. Herbelin, unpublished data), both strains being totally free of iNKT cells. In addition, pharmacological treatments (16–19) as well as overexpression of CD1d in Langerhans islets (15) prevented autoimmune diabetes in NOD mice.

In this study, we report for the first time that protection against diabetes was achieved in NOD.C3H 6.VIII congenic mice in which the frequency and number of iNKT cells were half those of normal NOD mice. The reduction, seen in both the thymus and the periphery (including islet infiltrates) is likely an intrinsic developmental defect of the iNKT lineage that affects the CD4+ but not the DN subpopulation, resulting in a significant decrease in the CD4+/DN ratio. This may be due to a reduced generation of CD4+ iNKT cells or an increased differentiation of CD4+ precursors into DN cells (2, 3). The decrease in iNKT cell number in the thymus shows that the perforin deficiency is neither due to a hampered migration from the thymus, nor to a modification in cell survival. The CD1d molecule is crucial for iNKT cell development, and down-modulation of its expression may alter their production (32, 33). However, CD1d expression on double positive thymocytes and peripheral lymphocytes was strictly identical in NOD and 6.VIII mice (data not shown).

It remains unclear what determines the CD4 vs DN development of iNKT cells. A stochastic decision or a choice directed by different TCR affinities for ligands has been proposed. In the human, CD4+ and DN iNKT cell subsets represent functionally distinct lineages with marked differences in their profile of cytokine secretion and pattern of expression of cell surface molecules (34, 35). Such differences between the two subsets have not been found in mice. However, according to our results, homeostasis of the two subsets appears to be independently regulated, compared with that of CD4+ iNKT cells involving genes of the Idd6 locus.

The capacity of iNKT cells of 6.VIII congenic mice to secrete IL-4 and IFN-γ upon stimulation by α-GalCer was not different from that of control NOD mice. Thus, the cytokine production decrease in 6.VIII mouse serum appears to result from a numerical defect.

Our analysis also provided further information about the influence of the NOD background on iNKT cells. First, the level of CD69 expression was largely decreased on NOD mouse cells from the thymus and spleen, revealing incomplete maturation (Ref. 36 and K. Benlagha, unpublished data). However, the almost normal level of CD69 in the liver may indicate either that the liver is a privileged site of iNKT cell maturation, or migration to the liver favors mature iNKT cells. Second, the relative proportion of Vβ7+ iNKT cells, shown to have the highest affinity for Ag (14), was higher in NOD than in nonautoimmune mice.

Whether iNKT cells play a role in the protection afforded by C3H Idd6 cannot be inferred from our results. However, if iNKT cells contribute to this protection, it is not through normalization of their number or IL-4 and IFN-γ secretion. Beside immune deviation, different mechanisms of action have been put forward for iNKT cell-mediated immunoregulation of type 1 diabetes. In particular, cooperation between iNKT and CD4+ CD25+ regulatory T cells in the protection of autoimmune diabetes (37) and myasthenia (38) has been documented. Yet, these studies involved α-GalCer activated iNKT cells, and one may question whether these conclusions would prove relevant under more physiological conditions. It would be interesting to investigate the possible relationship between CD4+CD25+ regulatory T cells and iNKT cells in 6.VIII mice that show a severe deficiency in iNKT cells while their CD4+CD25+ regulatory T cells exert enhanced inhibitory activity (26).

The increased proportion of the DN cell subset among the iNKT population in 6.VIII mice may suggest a role of DN iNKT cells in protection from diabetes, an effect that would be hindered in NOD mice due to the presence of opposing cells or their products. Indeed, DN iNKT cells were able to confer protection against tumor growth only when used purified, since CD4+ iNKT cell-produced IL-4 antagonized their ability to mediate IFN-γ-dependent tumor rejection (39). Recently, Cain et al. (40) reported that iNKT cell-produced IFN-γ was necessary for driving in vivo the inhibition of diabetogenic T cells in NOD mice, by modifying the maturation and function of IFN-γ-responsive DC.

Importantly, our results allowed an improved localization of the interval on chromosome 6, distinct from the NK complex, which influences the number and function of iNKT cells. Analyzing three subcongenic strains, we now show the iNKT cell abnormalities in 6.VIII mice due to the presence of opposing cells or their products. Indeed, DN iNKT cells were able to confer protection against tumor growth only when used purified, since CD4+ iNKT cell-produced IL-4 antagonized their ability to mediate IFN-γ-dependent tumor rejection (39). Recently, Cain et al. (40) reported that iNKT cell-produced IFN-γ was necessary for driving in vivo the inhibition of diabetogenic T cells in NOD mice, by modifying the maturation and function of IFN-γ-responsive DC. Importantly, our results allowed an improved localization of the interval on chromosome 6, distinct from the NK complex, which influences the number and function of iNKT cells. Analyzing three subcongenic strains, we now show the iNKT cell abnormalities in 6.VIII mice due to the presence of opposing cells or their products. Indeed, DN iNKT cells were able to confer protection against tumor growth only when used purified, since CD4+ iNKT cell-produced IL-4 antagonized their ability to mediate IFN-γ-dependent tumor rejection (39). Recently, Cain et al. (40) reported that iNKT cell-produced IFN-γ was necessary for driving in vivo the inhibition of diabetogenic T cells in NOD mice, by modifying the maturation and function of IFN-γ-responsive DC.

FIGURE 8. Genetic map of Idd6 locus on murine chromosome 6. The intervals introgressed into different Idd6 congenic strains are depicted.
has not been published yet. All these observations concur to show that genes involved in the protection against diabetes are located on distal chromosome 6, and absent from the NK complex region.

Collectively, our data show that protection against diabetes in \textit{Idd6} congenic NOD mice did not result from a correction of iNKT cell number or capacity to produce IL-4 and IFN-\gamma upon stimulation, and underscore a relationship between \textit{Idd6} and control of CD4\textsuperscript{+} iNKT cell development. Our data point to a more complex genetic control of iNKT cell defects in the NOD mouse and suggest that multiple loci effects may be required to link iNKT cell defects to type 1 diabetes and more generally to autoimmunity.

Acknowledgments

We thank Diane Mathis, Christophe Benoist, and Henri-Jean Garchon for the gift of mice, Kirin Brewery (Gumma, Japan) for providing \(\alpha\)-GalCer, and Laetitia Breton for animal care.

Disclosures

The authors have no financial conflict of interest.

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