corrections

Experimental verification of the quasi-unit-cell model of quasicrystal structure

Paul J. Steinhardt, H.-C. Jeong, K. Saitoh, M. Tanaka, E. Abe & A. P. Tsai

Nature **396,** 55–57 (1998)

Figure 3 of this Letter wrongly shows all cobalt sites as yellow filled circles, indicating that they lie on the same $c = 0$ level, whereas the two lower sites in the figure were intended to be open circles, indicating cobalt sites on the $c = 1/2$ level. A corrected version of the figure can be found on *Nature*'s website.

Extreme Th1 bias of invariant Vα24JαQ T cells in type 1 diabetes

S. Brian Wilson, Sally C. Kent, Kurt T. Patton, Tihamer Orban, Richard A. Jackson, Mark Exley, Steven Porcelli, Desmond A. Schatz, Mark A. Atkinson, Steven P. Balk, Jack L. Strominger & David A. Hafler

Nature **391,** 177–181 (1998)

In this Letter, we reported that invariant Vα24JαQ T cells from monozygotic diabetic twins/triplets were reduced in number and produced only interferon-γ on appropriate stimulation, whereas those cloned from at-risk non-diabetic twins/triplets and controls produced both interferon-γ and interleukin(IL)-4. We also reported (see our Fig. 4) that 50% (7/14) of high-risk diabetes non-progressors had markedly raised levels of serum IL-4, as measured by ELISA. However, we now find that measurement of serum IL-4 by ELISA is confounded by the presence in some serum samples of a heterophile-like substance(s) that gives false positive estimations for IL-4 by crosslinking the capture and detection antibodies used in the assay[1,2]. This is not the case when IL-4 is measured in the tissue-culture medium of *in vitro* activated T cells. Details will be published once the source of error is determined, a method for accurate measurement of serum IL-4 is established, and the apparent association of production of the heterophile substance and/or IL-4 with diabetic non-progression has been clarified. □

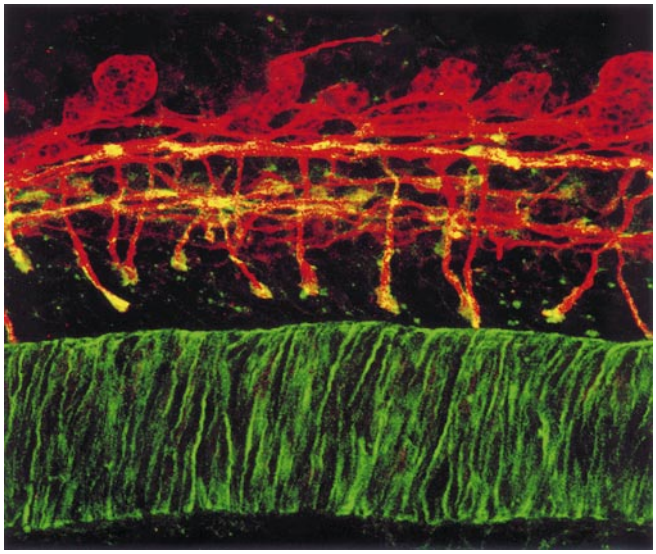
1. Redondo, M. *et al. Diabetes* **48** (suppl. 1) Abstr. (1999).
2. Ellis, T. *et al. Diabetes* **48** (suppl. 1) Abstr. (1999).

In vivo regulation of axon extension and pathfinding by growth-cone calcium transients

Timothy M. Gomez & Nicholas C. Spitzer

Nature **397,** 350–355 (1999)

The cover image on this issue should have been orientated as shown here to conform with the location of the notochord as described in the cover caption. □



The role of mat-forming diatoms in the formation of Mediterranean sapropels

Alan E. S. Kemp, Richard B. Pearce, Itaru Koizumi, Jennifer Pike & S. Jae Rance

Nature **398,** 57–61 (1999)

In this Letter, the legend to Fig. 2 has the descriptions switched for the last two panels. The image shown in Fig. 2c is in fact of *H. hauckii*, with scale bar 20 μm, and that in Fig. 2d is of *P. calcar-avis*, with scale bar 10 μm.

type (+/+) sibling of the same sex from the same litter. Locomotion experiments were run by an observer blind to the genotype of the animals being tested. Tests were run from 10 a.m. to 2 p.m. Data are presented as mean ± s.e.m. Statistical analysis was performed using ANOVA ($^* P < 0.05$ mutant compared with wild type).

Self-administration. Adult male mice (wild-type and mutant siblings of parents backcrossed 3 generations to C57 B1/6 inbred mice) were implanted with a silastic catheter in the jugular vein under halothane anaesthesia and tested in operant cages equipped with two nose-poke detectors, one active, the other inactive (V. Deroche *et al.*, manuscript in preparation). All mice were first trained with cocaine (0.8 mg kg^{-1} per injection, delivered in 50 μl per 2 s, with 20-s time-out period) under fixed-ratio (FR) 1 for 2–4 sessions, then under FR2 until stable baseline was reached. Spontaneous nose-poke behaviour, measured in naive, sham-operated mice when both detectors were inactive, was low and non-discriminatory (2.6 ± 0.6 per h), and did not differ between $\beta2^{-/-}$ mice and wild-type mice (not shown). The baseline for each mouse was defined as 3 consecutive sessions with less than 30% deviation from the mean and at least 75% active-specific response. During 5 consecutive daily sessions, cocaine was replaced with nicotine (0.03 mg kg^{-1} per injection, delivered in 50 μl per 2 s, 20-s time-out period, under FR 2 schedule) in wild-type mice ($n = 5$) and $\beta2^{-/-}$ mice ($n = 5$). In a second group of wild-type mice ($n = 5$), cocaine was replaced with saline, forcing the operant responding to extinction. Cocaine and nicotine bitartrate (Sigma) were freshly dissolved in saline before each experiment.

Equilibrium binding. Receptor autoradiography was done as described[19–21]. Briefly, following 30 min preincubation in the appropriate buffer, 14-μm coronal brain sections from wild-type and β2-mutant mice were incubated at room temperature for 120 min with 5 nM [^{3}H]WIN35,428 (84.5 Ci mmol^{-1}; NEN)[19] or for 60 min with either 1.5 nM SCH23390 (70 Ci mmol^{-1}; NEN)[20] or 3 nM raclopride (82.4 Ci mmol^{-1}; NEN)[21]. Slides were washed in ice-cold buffer twice for 1 min (WIN35,428) or for 5 min (SCH23390 or raclopride), and exposed to hyperfilm together with appropriate standards ([^{3}H]microscale; Amersham). Nonspecific labelling was determined in the presence of 30 μM cocaine for the dopamine transporter and 1 μM (+)butaclamol for D1 and D2. Siblings of the same sex and litter were used for all experiments (n, 4–6 per group).

Cyclase and tyrosine hydroxylase assays. Dopamine-stimulated cyclase activity was measured in striatal homogenates from wild-type and β2-mutant siblings of the same sex and same litter by following published protocols[22] and using the Amersham Biotrak scintillation proximity assay to determine cAMP levels. For each animal ($n = 6$ per group), the striatum from one side of the brain was used for dopamine-stimulated cyclase assays, and the striatum from the other side of the brain was used to measure tyrosine hydroxylase activity as described[23]. Briefly, after homogenization, samples were incubated with [^{3}H]tyrosine and tetrahydrobiopterin. End products were separated from unreacted [^{3}H]tyrosine by treatment with activated charcoal. Results are reported as the number of counts incorporated minus background per μg protein assayed.

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Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy form Mary Sheehan at the London editorial office of *Nature*.

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Extreme Th1 bias of invariant Vα24JαQ T cells in type 1 diabetes

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Type 1 diabetes (insulin-dependent diabetes mellitus, IDDM) is a disease controlled by the major histocompatibility complex (MHC) which results from T-cell-mediated destruction of pancreatic β-cells[1]. The incomplete concordance in identical twins and the presence of autoreactive T cells and autoantibodies in individuals who do not develop diabetes suggest that other

abnormalities must occur in the immune system for disease to result[2,3]. We therefore investigated a series of at-risk non-progressors and type 1 diabetic patients (including five identical twin/triplet sets discordant for disease). The diabetic siblings had lower frequencies of $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ T cells compared with their non-diabetic sibling. All 56 $V\alpha24J\alpha Q^+$ clones isolated from the diabetic twins/triplets secreted only interferon (IFN)-γ upon stimulation; in contrast, 76 of 79 clones from the at-risk non-progressors and normals secreted both interleukin (IL)-4 and IFN-γ. Half of the at-risk non-progressors had high serum levels of IL-4 and IFN-γ. These results support a model for IDDM in which Th1-cell-mediated tissue damage is initially regulated by $V\alpha24J\alpha Q^+$ T cells producing both cytokines; the loss of their capacity to secrete IL-4 is correlated with IDDM.

The discovery of Th1 and Th2 subsets of $CD4^+$ T cells has helped to explain the cellular basis for the diversity of T-cell responses in autoimmunity[4]. Th1 cells promote inflammatory cellular immune responses and are biased towards secretion of IFN-γ, tumour-necrosis factor (TNF)-β and IL-2. Th2 cells are biased towards secretion of interleukins 4, 5, 6, 10 and 13, induce humoral immunity, and inhibit Th1 responses. Lymphocyte cytokine production in type 1 diabetes is known to exhibit a bias towards the Th1 cytokine IFN-γ (ref. 5), but the cellular mechanisms integrating the drive to Th1 or Th2 effector cell differentiation are poorly understood. In the mouse, one mechanism by which Th2 rather than Th1 T-cell bias may be promoted is by activation of invariant (no N/P nucleotide additions within the CDR3 of the *TCRA* gene) $V\alpha14J\alpha281$ TCR^+ $NK1.1^+$ T cells capable of early secretory bursts of IL-4 and IFN-γ. The ligand for this family of T cells is CD1.1 on the surface of antigen-presenting cells[6,7].

$CD4^-CD8^-$ T cells in humans expressing the invariant $V\alpha24J\alpha Q$ T-cell antigen receptor (TCR) which has close sequence homology to the murine $V\alpha14J\alpha281$ TCR have been described[8]. To determine whether there could be a relationship between the number of circulating $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ T cells and type 1 diabetes, we did a frequency analysis on a set of type 1 diabetic discordant monozygotic twins and triplets. The numbers of circulating $CD4^-$ $CD8^-$ $V\alpha24J\alpha Q^+$ T cells in diabetes-free twins/triplets were compared with those present in their siblings with disease. The percentage of circulating invariant $CD4^-CD8^-$ $V\alpha24J\alpha Q$ T cells could be determined by multiplying the frequency of invariant $V\alpha24J\alpha Q$ sequences present in the total $CD4^-CD8^-$ $V\alpha24^+$population by the percentage of $CD4^-CD8^-$ $V\alpha24^+$ T cells, as measured by flow cytometric analysis (Table 1). No $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ T cells were detected in three diabetics, despite at least three sorting attempts for each subject. The percentage of $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ T cells in a previously disease-free diabetic twin (patient 6A; Table 1), studied during the week of IDDM diagnosis, was similar to that in the long-term IDDM twin and in the other diabetics. In all sets of family pairings, the IDDM sibling had markedly lower percentages of $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ T cells ($P = 0.015$, paired sign test using only the discordant twins/triplets data).

To determine whether human $V\alpha24J\alpha Q^+$ T cells were functionally altered in type 1 diabetics and those at risk for the disease, single $CD4^-/CD8^-$ mononuclear cells expressing $V\alpha24^+$ TCR were cloned. The initial analysis was carried out on clones generated from the IDDM non-progressing member of a sibling pair, subject 7A (Table 1). All clones expressed the invariant $V\alpha24J\alpha Q$ junctional sequences conserving the germ-line-encoded amino acids Vα24 (-CVVS:) and JαQ (:DRGST-). Eight of ten clones were $V\beta11^+$ and two were $V\beta13^+$. All of the clones were $CD4^-$ and uniformly negative when stained for CD8 β-chain. Surface expression of $CD8\alpha\alpha^+$ appeared to reflect the activation state, as staining for this marker reverted to negative 2–3 weeks post-stimulation. All T-cell clones expressed the human homologue of the murine NK1.1 molecule, NKR-P1A (ref. 9), and the C-type lectins encoded by the natural killer (NK) locus, CD69 and CD94 (data not shown).

CD1d restriction was assessed by co-cultivating $V\alpha24J\alpha Q^+$ T-cell clones with C1R cells transfected with a CD1d or control expression vector[10]. A T-cell clone (4.2) with a non-invariant TCR α-chain ($V\alpha24N3J\alpha6$) was included as a negative control. All T-cell clones, except 3.5, 3.8 and the control clone 4.2, specifically proliferated in response to the CD1d transfectant of CIR (Fig. 1a). All of the clones except 3.5 and 4.2 secreted IL-4 and IFN-γ in a CD1d-specific manner (Fig. 1b, c). Clone 3.5 secreted only IFN-γ in response to CD1d (Fig. 1). The fine specificity of the clones for CD1d was tested by using C1R targets transfected with CD1a, CD1c, CD1d or vector alone. Only CD1d-expressing target cells specifically stimulated each of the $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ clones, as assessed by IL-4 and IFN-γ secretion (Fig. 2).

A panel of $V\alpha24J\alpha Q^+$ T-cell clones was then raised from: (1) the twins/triplets discordant for type 1 diabetes (Table 1); (2) from an additional four at-risk non-progressors with raised serum IL-4 levels (see below); and (3) two haplotype (DR3/DR2 and DR4/DRX)-matched normal controls. Twenty-five out of 28 clones raised from the at-risk non-progressors among the discordant twins/triplets secreted both IL-4 and IFN-γ ($>10\ pg\ ml^{-1}$) on stimulation with anti-CD3 (Fig. 3a). The other three clones produced only IFN-γ. Unlike the other non-progressing twins, only one clone from triplet 1A secreted moderate amounts of IL-4 when stimulated. Only a single attempt to generate clones from this subject was made owing to subsequent entry into a clinical trial. All of the 56 clones raised from the diabetic twins/triplets secreted only IFN-γ with anti-CD3 stimulation, and diabetic twins 4B and 5B had no identifiable $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ T cells (Table 1). There was no difference in the proliferative response to anti-CD3 between the clones raised from diabetics or other subjects (data not shown). The new onset type 1 twin 6A (Table 1) had 9/9 $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ T-cell clones that secreted only IFN-γ (Fig. 3b). This suggested that the Th1 phenotype seen in the new-onset twin was not related to duration of diabetes but occurred before, or concurrently with, the

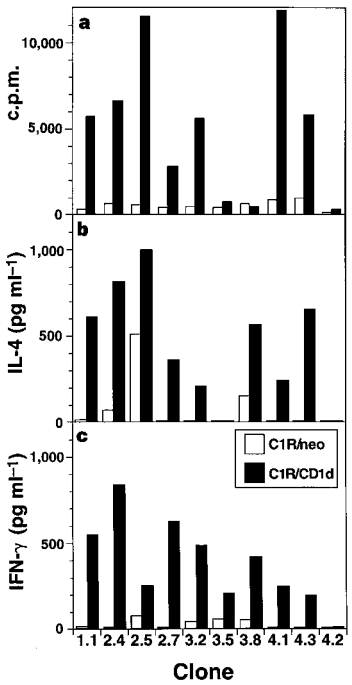

Figure 1 $CD4^-CD8^-$ $V\alpha24J\alpha Q$ T-cell clones respond to C1R/CD1d transfectants specifically. $V\alpha24J\alpha Q$ T cell clones (1.1, 2.4, 2.5, 2.7, 3.2, 3.5, 3.8, 4.1 and 4.3) and control clone 4.2 ($V\alpha24N3J\alpha6$), all at 5×10^4 per well, were stimulated with fixed C1R/CD1d or C1R/neo transfected cells at 5×10^4 per well. PMA ($1\ ng\ ml^{-1}$) was added. **a**, Proliferation was measured by tritiated thymidine incorporation at 72 h; **b**, secreted IL-4, and **c**, IFN-γ were assayed by ELISA at 48 h. All clones were from subject 7A. One of three representative experiments is shown.

onset of overt disease. As individuals discordant for the development of type 1 diabetes were genetically identical, the question remains as to what environmental factor(s), if any, may have triggered the decreased frequency and cytokine shift of the Vα24JαQ T cells.

An additional set of 33 clones was generated from four at-risk non-progressors (see below and Fig. 4), and 18 clones were raised from MHC haplotype-matched controls (Fig. 3b). Clones raised from these subjects were phenotypically similar to the diabetes-free twins and a series of invariant Vα24JαQ$^+$ T-cell clones previously described[10,11] Thus, all Vα24JαQ T-cell clones raised from type 1 patients showed an extreme Th1 bias, making them incapable of providing the IL-4 necessary for initiation of Th2 responses. In fact, unopposed IFN-γ secretion could augment or initiate a Th1-dominated cellular attack on pancreatic β-cells[12,13]

Our functional studies on Vα24JαQ$^+$ T cells from discordant twins/triplets, at-risk non-progressors and controls suggested that these two groups had polarized cell-mediated immune responses. We therefore tested serum IL-4 and IFN-γ in 14 at-risk IDDM non-progressors who had remained IDDM-free despite a 50% risk of developing diabetes during the study[14]. This cohort was defined by having remained healthy despite five or more years follow-up after diagnosis of type 1 diabetes in a first-degree relative and being positive for islet-cell antibodies (ICA$^+$) with any two of the following autoantibodies: anti-GAD, anti-IA2 or anti-insulin autoantibodies. Seven of 14 type 1 non-progressors had markedly raised levels of serum IL-4 (Fig. 4), six of whom also had raised IFN-γ (0.2–35 ng ml^{-1}). Despite the increase in cytokines in the serum from

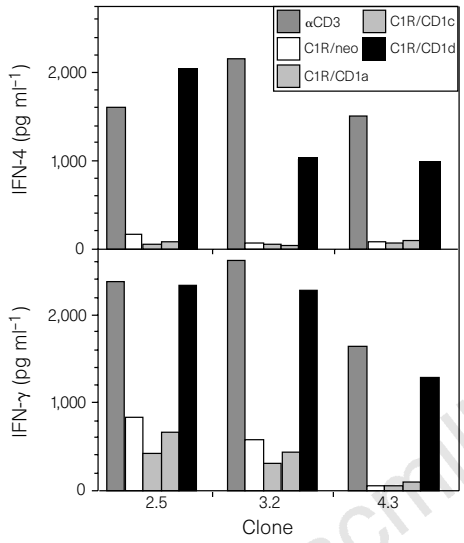

Figure 2 Specificity of three CD4$^-$CD8$^-$ Vα24JαQ T-cell clones for CD1 isoforms. Clones 2.5, 3.2 and 4.3 were co-cultivated with the following C1R transfectants: C1R/neo, C1R/CD1a, C1R/CD1c, and C1R/CD1d, as for Fig. 1: top, secreted IL-4; bottom, secreted IFN-γ. In addition, clones were activated with plate-bound anti-CD3 or immunoglobulin control.

Table 1 Frequency of CD4$^-$CD8$^-$ Vα24JαQ T cells from IDDM and disease-free siblings

Twins/triplets	DN (%)*	% DNVα24$^+$ in total lymphocytes	Vα24JαQ DN sequence frequency	Vα24JαQ (%)
1A/IL-4$^+$	0.74	0.04	20/22	0.036
1B/IDDM	0.95	0.01	10/19	0.005
1C/IDDM	0.76	0.04	9/22	0.016
2A	2.1	0.37	9/10	0.33
2B/IDDM	3.1	0.025	31/31	0.025
3A	1.1	0.04	8/12	0.027
3B/IDDM	1.89	0.01	5/15	0.003
4A	1.21	0.02	4/13	0.006
4B/IDDM	0.31	0.006	0†	0
5A	0.58	0.06	8/12	0.04
5B/IDDM	0.98	0	0†	0
6A/newIDDM	0.89	0.03	7/26	0.008
6B/IDDM	2.62	0.03	8/23	0.01
Brother/sister				
7A/IL-4$^+$	2.54	0.03	8/12	0.017
7B/IDDM	1.08	0.005	0/18	0

The frequency of Vα24JαQ TCR sequences was determined by sorting all CD4$^-$CD8$^-$ αβTCR$^+$ T cells, amplifying all Vα24 transcripts and sequencing the TCR CDR3 region; the percentage of cells that were invariant CD4$^-$CD8$^-$ Vα24JαQ in total mononuclear cells was calculated by multiplying the sequence frequency by the CD4$^-$CD8$^-$ Vα24$^+$ percentage of total mononuclear cells, as determined by flow cytometry. IL-4$^+$ indicates a subject with high serum IL-4. * DN, CD4$^-$CD8$^-$. † No Vα24 PCR products were detected in three attempts.

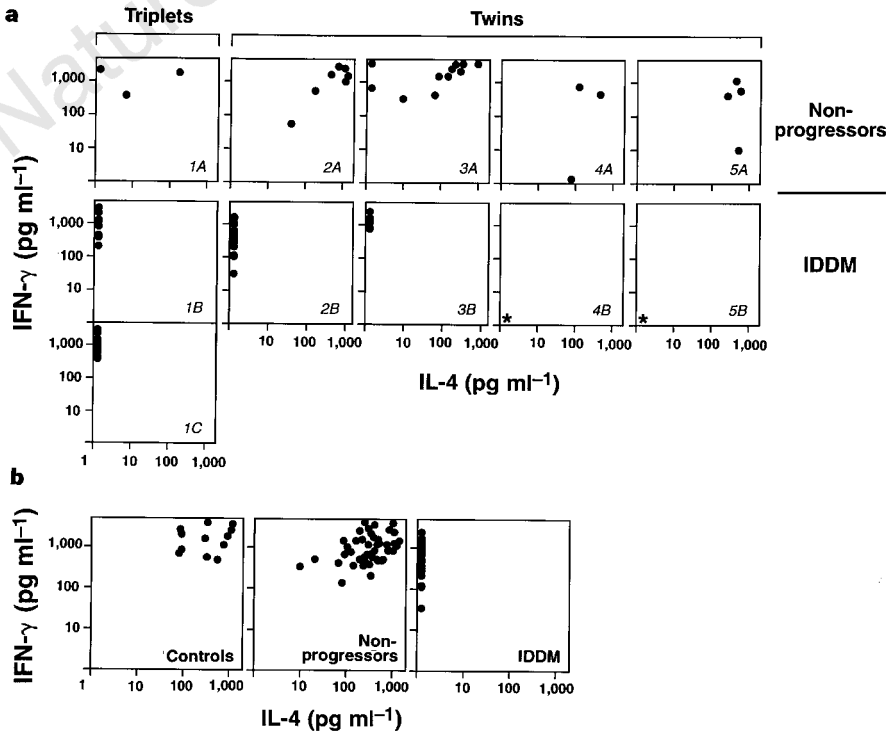

Figure 3 IL-4 and IFN-γ secretion profiles of CD4$^-$ CD8$^-$ Vα24JαQ T-cell clones raised from monozygotic twins and triplets discordant for IDDM. **a**, Plate-bound anti-CD3 or control immunoglobulin was used to stimulate individual clones and secreted IL-4 and IFN-γ was assayed at 4 h. The pattern of cytokine secretion was similar at 24 h. The CDR3 T-cell receptor sequences were determined and the surface phenotype was confirmed by flow cytometry for every clone (data not shown). Asterisks, twins with IDDM (4B and 5B) had no detectable Vα24JαQ T cells in two attempts to sort from 20 million PBMCs (Table 1). **b**, CD4$^-$CD8$^-$ Vα24JαQ T-cell clones from 4 IDDM non-progressing subjects (Fig. 4), two control (DR2/DR3 and DR4/DRX) and five IDDM patients (triplets 1B and 1C, twins 2B and 3B, and new-onset IDDM twin 6A), were assayed as described in **a**.

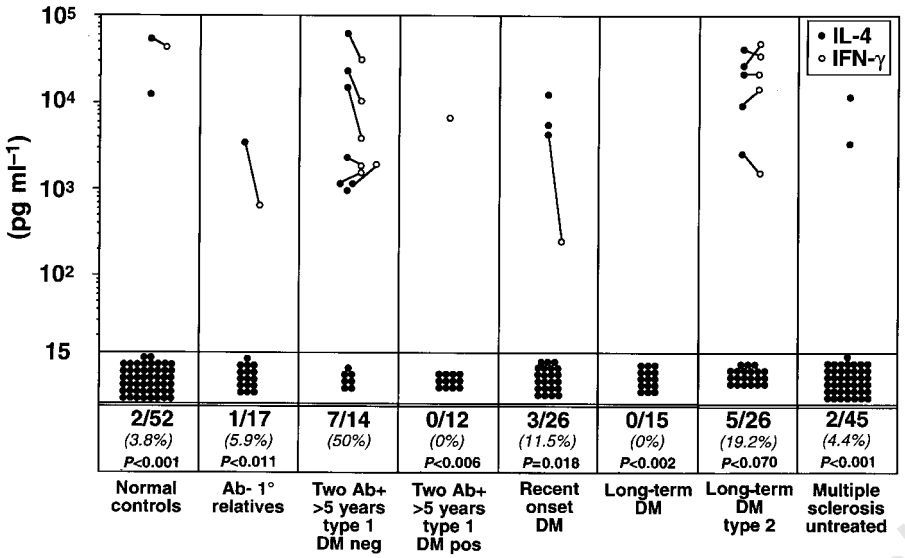

Figure 4 Serum IL-4 and IFN-γ in type 1 diabetes. Sera were collected and assayed for IL-4 and IFN-γ by capture ELISA, the detection minimum for which was 15 pg ml^{-1}. Raised cytokines were detected in 7/14 members of the at-risk IDDM non-progressor cohort. Lines connecting symbols indicate samples in which both IL-4 and IFN-γ were detected; no IFN-γ was detected in six subjects with raised IL-4. *P, Two-tailed Fisher exact test comparing high IL-4 frequency in each cohort with at-risk IDDM non-progressors.

seven of fourteen non-progressors, all seven IL-4$^+$ individuals have remained healthy with no evidence of chronic infectious or atopic/allergic illnesses. Both cytokines in the remaining seven were below the detection limit of our enzyme-linked immunosorbent assay (ELISA; 0.015 ng ml^{-1}). Five of 14 individuals in this group were found to have the strongly protective MHC allele DQB1*0602 and therefore are not at the same risk of progression as the remaining nine members of this cohort[15]. Three of these five individuals had raised serum IL-4 and IFN-γ levels, in contrast to our finding that IL-4 was undetectable in the serum (before or after diagnosis of type 1 diabetes) in 12 individuals with identical autoantibody status who developed IDDM after five or more years of follow up (Fig. 4).

Raised cytokines were also detected in archival serum samples obtained from 3/23 individuals at the time of diagnosis of type 1 diabetes and in 5/26 type-2 diabetics who did not have autoantibodies or a family history of type 1 diabetes (Fig. 4). When compared with normals, antibody-positive first-degree relatives, recent-onset diabetics, long-term diabetics (IDDM >2 years), autoantibody-negative first-degree relatives or untreated patients with multiple sclerosis (MS), the frequency of serum IL-4$^+$ individuals was significantly raised in the non-progressor consort (Fig. 4). The authenticity of the IL-4 detected was confirmed independently by using another set of ELISA antibodies, by binding to soluble recombinant IL-4 receptor produced in insect cells, and by western blot (data not shown).

Our results demonstrate a relationship between elevated serum-IL-4 levels and resistance to the progression of an autoimmune disorder. Prolonged hyperglycaemia as an explanation for the absence of IL-4 in type 1 diabetics is less likely because IL-4 was detected in the serum of type 2 diabetics. Increased IL-4 was not an absolute predictor of IDDM resistance as only half of the resistant cohort had raised serum IL-4, as did 3/23 diabetics at or near the time of diagnosis.

In the non-obese diabetic (NOD) mouse, there is evidence that IL-4 exerts a dominant-negative effect on the progression to IDDM[16–18]. Differentiation of T cells into IL-4 secreting Th2 effector cells requires IL-4 priming[4]. Although this proposed function for NK1.1$^+$ T cells was not obligatory for all Th2 immune responses[6,19,20], T-cell IL-4 secretion was markedly diminished in a CD1 knockout background[20–22]. Fewer NK 1.1$^+$ T cells were found to be present and were less frequent before the onset of disease in several murine methods of autoimmunity[6,7,23–25]. In these models, autoimmunity was accelerated by depletion of NK 1.1$^+$ T cells and delayed by generating mice transgenic for the Vα24Jα281 TCR. Diabetes was also prevented in the NOD mouse by adoptive transfer of a population harbouring the NK1.1-like class of T cell[26].

In summary, type 1 diabetes is associated with an extreme Th1 phenotype for Vα24JαQ$^+$ T cells and a decrease in their circulating frequency. Our results suggest that there is a strong link between Vα24JαQ$^+$ T cells and human type 1 diabetes; this indicates that they may be functionally related to the resistance or progression of this autoimmune disease in humans. □

Methods

Antibodies and phenotypic analysis of T cells. Flow cytometry experiments were done on FACScaliber and FACS Vantage instruments (Becton Dickinson). Monoclonal antibody (mAb) DX1 was a gift from L. Lanier; anti-CD4, anti-CD8 and anti-panTCR were from Becton Dickinson; anti-Vα24, anti-CD8β, anti-CD56, anti-CD16 and anti-p58CD158 (NK workshop mAbs GL183 and EB6) were from Immunotech; anti-CD69 and anti-CD94 were from Pharmingen.

CDR3 TCR sequencing. Total CD4$^-$CD8$^-$ Vα24JαQ CDR3 sequences were amplified by reverse transcription followed by polymerase chain reaction (RT-PCR) using Vα24 and constant-region-specific primers as described[8], and cloned using a Stratagene pCR-Script kit. Individual T-cell clones TCR transcripts were amplified by RT-PCR. Sequences of the plasmid and PCR DNA products were determined directly on an ABI 373A Automated DNA Sequencer.

Cell culture and cytokine assay. Single-cell sorts were grown with a mixture of irradiated (5,000 rad) allogeneic feeders at 50,000 per well and 721.221 cells at 5,000 per well and supplemented with 1 μg ml^{-1} PHA-P, IL-2 and IL-7 each at 10 U ml^{-1}, then propagated as described[27]. Clones positive for Vα24 and NKR-P1A by flow cytometry and a Vα24JαQ CDR3 TCR sequence were assayed for cytokine secretion. Cells were stimulated (25,000 per well) with plate-bound anti-CD3 (1 μg ml^{-1}; Immunotech) or control isotype antibody (Sigma) for 4, 8 or 24 h. Supernatants were assayed for IL-4 and IFN-γ by capture ELISA, and after 24 h, 1 μCi per well of [^{3}H]thymidine was added and incorporation measured as described[27].

CD1 restriction. Restriction experiments using CD1 isoform (CD1a, CD1c, CD1d and pSRα-neo vector alone) transfected C1R cells were done as described[10].

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Drosophila Shaking-B protein forms gap junctions in paired *Xenopus* oocytes

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In most multicellular organisms direct cell–cell communication is mediated by the intercellular channels of gap junctions. These channels allow the exchange of ions and molecules that are believed to be essential for cell signalling during development and in some differentiated tissues. Proteins called connexins, which are products of a multigene family, are the structural components of vertebrate gap junctions[1,2]. Surprisingly, molecular homologues of the connexins have not been described in any invertebrate. A separate gene family, which includes the *Drosophila* genes *shaking-B* and *l(1)ogre*, and the *Caenorhabditis elegans* genes *unc-7* and *eat-5*, encodes transmembrane proteins with a predicted structure similar to that of the connexins[3–9]. *shaking-B* and *eat-5* are required for the formation of functional gap junctions[8,10]. To test directly whether Shaking-B is a channel protein, we expressed it in paired *Xenopus* oocytes. Here we show that Shaking-B localizes to the membrane, and that its presence induces the formation of functional intercellular channels. To our knowledge, this is the first structural component of an invertebrate gap junction to be characterized.

The *shaking-B* (*shak-B*) locus was identified in *Drosophila* in two independent screens for behavioural mutants[11,12]. Two transcripts, referred to as *shak-B*(*neural*) (formerly *Passover*) and *shak-B*(*lethal*), which share five 3′ exons, encode proteins of relative molecular mass 43,000 to 44,000 (M_r 43K–44K)[3–5]. Both are predicted to have four transmembrane domains, two extracellular loops, and cytoplasmic amino and carboxy termini[3]. By comparing dye-coupling in wild-type and mutant flies, we have shown that the product of *shak-B*(*neural*) is essential for the function of the gap junctions at electrical synapses in the giant fibre system, the pathway that subserves the insect's escape response[10], and in some embryonic somatic muscle (R.A.B. *et al.*, unpublished data). These studies in *Drosophila* do not tell us whether Shak-B proteins are integral channel components of gap junctions or accessory proteins necessary for gap junctions to function. To distinguish between these possibilities we expressed *shak-B* RNAs in the *Xenopus* oocyte system, which has been used extensively to characterize the channel-forming ability of the vertebrate connexins[13].

RNAs encoding Shak-B(neural) and Shak-B(lethal) were micro-injected, either individually or together, into the vegetal hemisphere of single oocytes. We first checked whether the oocytes efficiently

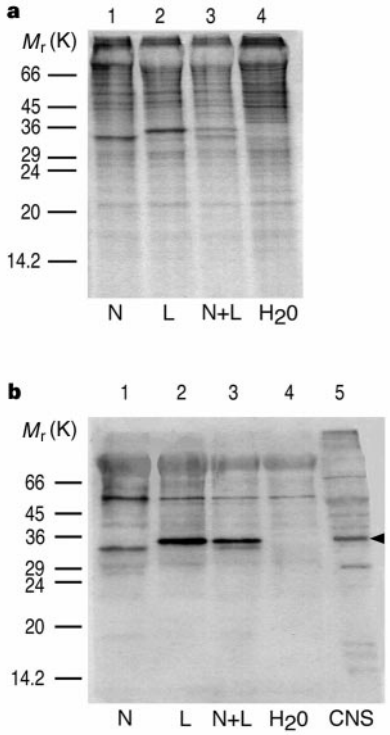

Figure 1 *shak-B* RNA is translated in the *Xenopus* oocyte expression system. **a**, Total radiolabelled proteins in membranes (single cell equivalent) prepared from oocytes 24 h after injection of [^{35}S] methionine (0.3 μCi) and 10 ng of *shak-B*(*neural*) RNA (N, lane 1), *shak-B*(*lethal*) RNA (L, lane 2), both RNAs (N + L, lane 3), or water (H_2O, lane 4). Bands unique to the RNA-injected cells (lanes 1–3) are Shak-B proteins. **b**, Western blot of proteins in membranes (half-cell equivalent) of RNA or water-injected oocytes (lanes 1–4, as in **a**) and in a homogenate of adult *Drosophila* nervous systems (CNS, lane 5). The blot was probed with Shak-B antiserum[10]. Labelled proteins (lanes 1–3) correspond in size to the proteins uniquely detected in **a** (lanes 1–3), and to native *Drosophila* Shak-B protein (lane 5, arrowhead). Positions of M_r markers are indicated on the left.

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