artificial cerebrospinal fluid containing 119 mM NaCl, 2.5 mM KCl, 1.0 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, and 10 mM glucose, equilibrated with 95% O_2 and 5% CO_2 (pH 7.3 to 7.4) at 21° to 23°C. In some experiments, picrotoxin (50 to 100 μM) was added to the bath solution with no effect on LTD (mean depression was 47.5 ± 10%, n = 10, in the absence of picrotoxin versus 45.5 ± 10%, n = 12, in the presence of 100 μM picrotoxin). Whole-cell patch-clamp recordings were obtained from CA1 pyramidal cells. The patch electrodes (5 to 10 megohms) contained 130 mM KCl, 0.4 mM $CaCl_2$, 1.1 mM EGTA, 1.0 mM $MgCl_2$, 5.0 mM NaCl, 10 mM potassium Hepes (pH 7.3), 2 mM Mg^{2+}–adenosine triphosphate, and 0.1 mM Na^+–guanosine triphosphate. Afferent fibers in the stratum radiatum were stimulated at 0.05 to 0.1 Hz with bipolar stainless steel electrodes. The holding potential was −70 mV. To elicit LTD, we applied a period of 5-Hz stimulation for 3 to 5 min under voltage-clamp or current-clamp conditions. Under current-clamp mode, the soma membrane depolarized by 15 to 25 mV during the train. We constructed summary graphs of EPSC amplitudes by normalizing data in 60-s epochs to the baseline EPSC recorded for 10 to 15 min at the start of each experiment. MCPG was purchased from Tocris Neuramin (Bristol, England), and DM-nitrophen from Calbiochem.

19. Ultraviolet (UV) illumination (Phillips 90-W quartz mercury high-pressure arc lamp), for 40 to 60 s in duration, was applied 15 min after obtaining the whole cell configuration. We estimated $[Ca^{2+}]$ to be >1 μM, on the basis of microcuvette measurements with fura-2 (*31*). We omitted Mg^{2+} from the intracellular solution because of its high affinity for DM-nitrophen (*32*). Evidence that UV photolysis elevated $[Ca^{2+}]$ includes induction of a Ca-activated K^+ current. Also, elevated $[Ca^{2+}]$ can participate in inducing LTD under appropriate conditions (*27*). Electrical stimulation of the Schaffer collaterals (3 min at 5 Hz) 5 to 8 min after photolysis of DM-nitrophen induced normal LTD (EPSC was reduced to 54.4 ± 2.8% of control; n = 3), indicating that the DM-nitrophen and UV light exposure did not inhibit LTD.
20. NMDA elicited inward currents (>100 pA) with typical voltage-dependent rectification. The D-APV (50 μM) decreased a late phase of the EPSC by 19 ± 5% at 25 ms (mean ± SEM; t = 3.2, $P < 0.02$, paired t test; n = 6).
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26. The sEPSCs were recorded on videotape for off-line analysis. Data were analyzed with the SCAN 4.0b program (J. Dempster, Dagan, Minneapolis, MN). Signals were compared with a threshold set above baseline noise (−1.5 to −2 pA), and samples were collected and stored when the threshold was exceeded. Noise artifacts and overlapping events have been excluded.
27. A train of short (50-ms) depolarizing steps to 0 mV at 5 Hz was applied to the postsynaptic neuron during ACPD application. This caused an irreversible presynaptic inhibition in all slices even after ACPD was washed out for 30 min (n = 7). The same train in the absence of ACPD had no effect on EPSC (n = 3). We also produced an irreversible depression of the EPSC by pairing ACPD with UV photolysis of DM-nitrophen (V. Y. Bolshakov and S. A. Siegelbaum, unpublished data), consistent with a role for increased $[Ca^{2+}]$ and providing a positive control for the experiment of Fig. 1E.
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16 February 1994; accepted 4 April 1994

Linkage Analysis of *IL4* and Other Chromosome 5q31.1 Markers and Total Serum Immunoglobulin E Concentrations

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Sib-pair analysis of 170 individuals from 11 Amish families revealed evidence for linkage of five markers in chromosome 5q31.1 with a gene controlling total serum immunoglobulin E (IgE) concentration. No linkage was found between these markers and specific IgE antibody concentrations. Analysis of total IgE within a subset of 128 IgE antibody–negative sib pairs confirmed evidence for linkage to 5q31.1, especially to the interleukin-4 gene (*IL4*). A combination of segregation and maximum likelihood analyses provided further evidence for this linkage. These analyses suggest that *IL4* or a nearby gene in 5q31.1 regulates IgE production in a nonantigen-specific (noncognate) fashion.

The immunogenetic mechanisms underlying heightened IgE responsiveness seen in the atopic diseases may be divided into two types, antigen (Ag)-specific and non-Ag–specific (*1*). The former is strongly influenced by *HLA-D*–encoded, major histocompatibility complex class II genes (*1*, *2*) and involves cognate T cell–B cell interaction. The latter, noncognate regulation of IgE, could involve primarily basophils, mast cells, and possibly other $Fc\epsilon RI^+$ cells, with supplemental involvement of T cells (*3*, *4*).

T helper lymphocytes, types 1 and 2 (T_H1 and T_H2), play a crucial role in facilitating the immune response (*5*, *6*). The expression of several diseases can depend on whether T_H1 or T_H2 lymphocytes predominate in response to an Ag challenge (*7*). The IgE antibody (Ab)–mediated atopic allergies provide a particularly good model for studying T_H2-associated diseases. The Ag-cognate interaction of B cells with T_H2 cells involves CD40-CD40L binding, B cell activation, and the release of IL-4 and IL-13 from the T_H2 cells. This process leads to Ig heavy chain class-switching to the ε isotype, resulting in specific IgE Ab responses (*6*, *8*, *9*). It appears that IL-5 facilitates IL-4–induced class-switching (*10*). Interleukin-4 is crucial for the development and functioning of T_H2 cells (*5*, *6*, *8*, *11*), including their ability to express IL-5, leading to eosinophilia (*11*). It has been shown that activated normal human basophils express and secrete IL-4, in a process that is facilitated by IL-3 (*3*). The basophils are able to interact with B cells through CD40-CD40L, leading to the production of IgE (*3*). This interaction is not Ag-driven and is, therefore, noncognate. The overall production of IgE by both cognate and noncognate pathways is reflected in the total serum IgE concentration, which can readily be measured as a quantitative trait.

Studies of twins have shown that total serum IgE concentration is largely determined by genetic factors (*12*). Family studies have favored recessive, dominant, or codominant models for a postulated major gene controlling total IgE (*1*, *13*, *14*), usually with a significant polygenic influence (involving multiple minor genes). Estimates of heritability of log[total IgE] from twin and family studies of Caucasoid subjects range from about 0.37 to 0.84 (*12*, *14*). Several family studies have provided evidence against linkage between a major gene for total IgE and *HLA* in chromosome 6p21.3 [reviewed in (*1*)]; also, the existence of an "atopy gene" in chromosome 11q13 is disputed (*15*).

The gene *IL4* has emerged as a major candidate for IgE responsiveness and atopy,

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with other candidate genes for atopic disease, including *IL5* and *IL13*, mapping within the "*IL4* cytokine-gene cluster" in chromosome 5q31.1, or within 5q31.2–q33 (Fig. 1). Therefore, we elected to test for linkage between log[total serum IgE] or log[serum IgE Abs] and several polymorphic genetic markers in and around 5q31.1–q33, with a primary focus on markers mapping within *IL4* itself and in, or close to, the *IL4* cluster. We also simplified the genetic complexity of the problem by studying large families from a genetically isolated farming community with a relatively uniform environment, namely the Pennsylvania Old Order Amish. Smoking, which has been associated with elevated total IgE (*16*), is relatively rare or light in the Amish; therefore, its effect on IgE should be negligible.

Eleven large Caucasian Amish families (170 subjects, 59% male) were selected on the basis of detectable serum IgE Ab to common inhalant allergens (*17*) in at least one child. All family members gave their informed consent. Analysis of eight DNA markers in chromosome 5q (Fig. 1) was performed (*18, 19*). Total IgE (*20*) and Ag-specific IgE Ab (*17*) concentrations were measured. Multiallergen IgE Abs were assayed to a composite of 20 common aeroallergens; IgE Abs were also assayed to the house-dust mites *Dermatophagoides pteronyssinus* (*Der p*) and *D. farinae* (*Der f*). We analyzed the marker and IgE data with the sib-pair method originally proposed by Haseman and Elston, as implemented in SIBPAL in the computer package S.A.G.E. (*21*). The quantitative traits investigated were log[total IgE], log[multiallergen IgE Ab], log[*Der p* IgE Ab], log[*Der f* IgE Ab], and log[mean *Der p*–*Der f* IgE Ab].

A total of 119 sibs [77 male (M), 42 female (F)], which provided 349 sib pairs, were analyzed from the 20 nuclear families comprising the 11 pedigrees. The mean age of these sibs was 22.9 ± 16.4 (SD) years, ranging from 4 to 79 years, with almost identical means, standard deviations, and ranges for both sexes. Males had higher geometric mean total IgE concentrations than females (126.5 compared with 70.3 ng/ml, $P = 0.05$). The mean age for the entire group of 170 subjects was 34.1 ± 23.1 (SD) years, ranging from 4 to 82 years, with males having twofold higher geometric mean total IgE concentrations than females (104.5 compared with 52.6 ng/ml, $P = 0.008$). Similar sex-associated trends were observed for multiallergen IgE Ab and the two mite-specific IgE Ab concentrations, but these differences were not significant. Thirty-five percent of the population (39% M and 30% F) was positive in the multiallergen test, and 21% (24% M and 17% F) was positive to one or both of the *Dermatophagoides* mites, predominantly *Der f*. These sex-associated differences are expected, because males (especially young males) are known to have significantly higher total IgE and to be significantly more atopic than females in the same age range (*1*). The log[total IgE] was only moderately well correlated with the log[multiallergen IgE Ab] ($r = 0.52$, $P = 0.0001$).

Table 1 (columns 4 to 6) shows the results for all 349 sib pairs for the eight markers in or near chromosome 5q31–q33 with log[total IgE], in which sex and age were included as covariates. Notably, *IL4-R1*, *IRF1*, *IL9*, *D5S393*, and *D5S399*, all located in 5q31.1, gave significant evidence for linkage. The loci mapping outside 5q31.1, namely *D5S404* on the centromeric side and *D5S210* and *CSF1R* on the

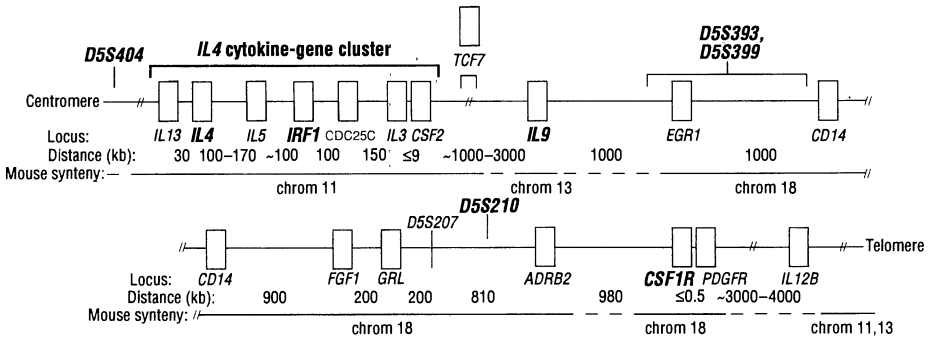

Fig. 1. A map showing genes of known physical location and certain polymorphic microsatellite markers in and around human chromosome 5q31.1–q33 (*24, 36*). Beside various interleukin genes, the map includes the following genes: *IRF1*, interferon regulatory factor–1, which encodes a transcription activator of *IFNA* and *IFNB* and other IFN-inducible genes; *CDC25C*, cell division cycle 25; *CSF2*, granulocyte-macrophage colony stimulating factor; *TCF7*, T cell–specific transcription factor–7; *EGR1*, early growth factor response–1; *CD14*, cell-Ag 14; *FGF1*, fibroblast growth factor–1 (acidic); *GRL*, lymphocyte-specific glucocorticoid receptor; *ADRB2*, the β_2-adrenergic receptor; *CSF1R*, colony stimulating factor–1 receptor; and *PDGFR*, platelet-derived growth factor receptor. Band 5q31.1 extends approximately from *IL13* to *CD14*. No recombination has been observed between *D5S393* and *D5S399* in this study, or as reported (*34*). The reported sex-averaged recombination distances, *D5S404* to *IL9*, *IL9* to *D5S399*, and *D5S399* to *CSF1R*, are 9.9 to 10.3 centimorgans (cM), 1.3 to 2.5 cM, and 14.7 to 26.8 cM, respectively (*34*). As shown, segments of various mouse chromosomes are syntenic to this region, including the *IL4* cytokine-gene cluster in mouse chromosome 11 (*34*). For methods, see (*37*).

Table 1. Sib-pair analyses for age- and sex-adjusted log[total IgE] for eight chromosome 5q markers in 11 Amish kindreds for the entire set of 349 pairs of full sibs and the set of 128 pairs of full sibs, all of whom have no detectable IgE antibody as determined from the multiallergen test.

Locus	No. of alleles†	Hetero-zygosity‡	349 pairs of sibs*			128 pairs of sibs with undetectable IgE Ab		
			π mean§	*t* statistic	*P* value‖	π mean§	*t* statistic	*P* value‖
D5S404	7 (7)	0.57 (0.73)	0.486	−0.75	0.23	0.479	0.10	0.54
IL4-R1	5 (10)	0.31 (0.49)	0.522¶	−2.47	0.0069	0.506	−4.65	0.000004
IRF1	4 (4)	0.55 (0.74)	0.506	−1.68	0.047	0.484	−3.27	0.00071
IL9	7 (9)	0.84 (0.80)	0.504	−2.08	0.019	0.475	−2.10	0.019
D5S393	7 (10)	0.86 (0.84)	0.510	−2.54	0.0058	0.492	−3.13	0.0011
D5S399	6 (8)	0.87 (0.80)	0.503	−2.90	0.0020	0.483	−3.24	0.00078
D5S210	7 (10)	0.77 (0.78)	0.493	−1.21	0.11	0.482	−1.00	0.16
CSF1R	7 (10)	0.81 (0.86)	0.494	−0.27	0.40	0.460	1.10	0.86

*For these analyses, there were 345 df, except for the *CSF1R* marker (336 df) for which we were unable to assign the alleles of one of the sibs unequivocally because of a mutation in the microsatellite repeat length at this locus. For the other set of analyses, there were 124 df in all cases. †The numbers of alleles found in our non-Amish studies or published studies (*34*) are shown in parentheses. ‡For each of the markers, we estimated the total heterozygosity contributed by the 20 nuclear families by combining data from the phenotypes of each parental mating, adjusted by the number of sib pairs. The heterozygosity reported for Caucasoid populations (*34*) is shown in parentheses. §Average proportion of marker alleles identical by descent (IBD) over all pairs of full sibs. ‖The variability in lod scores for the 5q31.1 markers (*IL4-R1* to *D5S399*) reflects the different information content of the markers as well as linkage to log[total IgE]. Omission of the covariates, age and sex, led to very little change in the *P* values. Because the sib-pair method assumes a normal distribution, it can be highly sensitive to the presence of outlying data points. In this context, it is worth noting that the log[total IgE] distributions did not differ significantly from normality. In addition, when all data points lying ≥3 SD from the mean of each distribution were removed, the significance was of an equal or greater order of magnitude for all the 5q31.1 markers. For example, when outliers were deleted, the *P* value for the IgE Ab⁻ subset for *IL4* became 0.000001. ¶Value is significantly different from 0.500

telomeric side, showed no significant evidence for linkage ($P > 0.1$). None of these eight markers showed significance ($P \geq 0.2$) for similar sets of analyses with any of the following IgE Ab traits: log[multiallergen IgE Ab], log[*Der p* IgE Ab], log[*Der f* IgE Ab], or log[mean *Der p*–*Der f* IgE Ab]. In further sib-pair analyses of log[total IgE] data, we included log[multiallergen IgE Ab] as an additional covariate (with age and sex). Our results generally showed slightly more significant *P* values than shown in Table 1 for all 349 pairs of sibs; for example, the *P* value for *IL4-R1* was 0.002. Our analyses, therefore, provide evidence of linkage of the 5q31.1 markers with log[total IgE], but not with log[IgE Ab] to common aeroallergens, suggesting genetic control on overall IgE production in a noncognate fashion.

The specific IgE Ab component, induced by means of Ag-cognate mechanisms, is markedly influenced by immune response (*Ir*) genes and by environmental exposure to allergens. Therefore, the total IgE phenotypes of atopic subjects contain a substantial amount of "noise" which reflects their variable IgE Ab profiles (*22*); this can be eliminated by removing these subjects. Therefore, we next excluded all sibs who had detectable IgE Ab by the multiallergen test and analyzed the remaining 128 "nonatopic," IgE Ab^- sib pairs. *IL4* and the neighboring marker, *IRF1*, now showed stronger evidence of linkage with log[total IgE] ($P = 0.000004$ and $P = 0.0007$, respectively; Table 1, columns 7 to 9, analyses of 128 pairs of sibs). Moreover, the overall consistency and significance of the associations for five markers in 5q31.1, as well as exclusion for markers outside this region, argue against this being a spurious result. We also analyzed the "atopic," IgE Ab^+ subset (66 sib pairs) and found no evidence of linkage for any of the chromosome 5 markers, either for log[total IgE] or log[multiallergen IgE Ab].

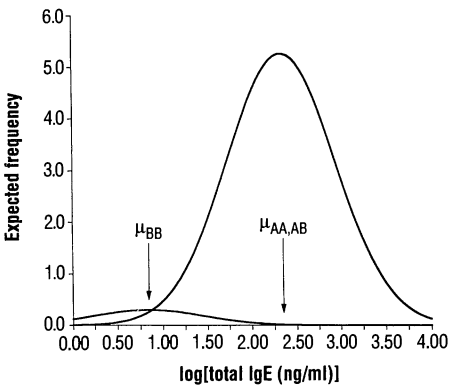

Fig. 2. Predicted distribution for log[total IgE] under the best-fitting two-distribution (dominant high IgE) model obtained from segregation analysis of all 170 Amish subjects. This major gene accounts for approximately 25% of the variance, similar to our previous reports (*14*). The model predicts that 94.6% of the population (genotypes AA and AB) would be drawn from a normal distribution with a mean log[total IgE (ng/ml)] $\mu_{AA,AB} = 2.34 \pm 0.12$ (SD), whereas 5.4% (genotype BB) would be drawn from a distribution with $\mu_{BB} = 0.85 \pm 0.40$ (SD). This model includes residual correlations among full sibs ($\rho_{SIB} = 0.21$), between spouses ($\rho_{SP} = -0.21$), and between parent and offspring ($\rho_{PO} = 0.03$), as well as fixed effects of age and sex ($\beta_{Age} = -0.008$ and $\beta_{Sex} = -0.28$).

To investigate further the linkage between a locus controlling log[total IgE], we carried out segregation analysis for all 170 Amish subjects under Class D regressive models, including sex and age as covariates, while estimating residual spouse-spouse, parent-offspring, and sib-sib correlations (*23*). Two-distribution models, either dominant high or dominant low log[total IgE], fitted the data significantly better than a single-distribution model ($\chi^2 = 7.95$ and 7.50 with 2 df; $P < 0.025$ in both cases). There was no evidence that the codominant model provided a better fit than the high or low dominant model ($\chi^2 = 0.15$ and 0.30 with 1 df; *P* not significant). The major gene for total IgE that we previously identified (*14*) had the same general characteristics. However, our "best" high dominant model (Fig. 2), like our previous model, suffers from a substantial overlap of the distributions, thereby complicating likelihood-based linkage analysis.

Maximum likelihood estimates, expressed as lod scores (logarithm of the likelihood ratio for linkage), for linkage of log[total IgE] to the eight markers are presented in Table 2. The two analyses in this table encompass slightly different subsets, as described in the table. All but the two outermost markers gave positive lods up to 1.84. These scores, by themselves, are not normally considered significant under the null hypothesis of no linkage (typically a lod ≥ 3.0 for a genome-wide screen). However, given the a priori hypothesis that *IL4* is a primary candidate gene, these lod scores for five 5q31.1 markers and *D5S210* (5q31.2) strongly suggest linkage.

Table 2. Lod scores for two-point analyses for linkage of log[total IgE] to eight markers in and around 5q31.1 on two subsets of 11 Amish kindreds under the high IgE dominant Mendelian model. Because of computational difficulty arising from a marriage loop, we performed two separate analyses, which required alternately excluding sets of double first cousins.

Marker	Lod at θ = 0.00*	
	Analysis 1	Analysis 2
D5S404†	−2.07	−2.37
IL4-R1	0.76	0.78
IRF1	0.76	0.75
IL9	1.12	1.08
D5S393	1.29	1.22
D5S399	1.45	1.39
D5S210	1.84	1.71
CSF1R†	−1.54	−1.88

*The lods for each of the markers under the best-fitting low IgE dominant and codominant models were very similar to those shown above, which probably reflects the marked overlaps between the phenotypic distributions. †The maximum likelihood estimates all peaked at 0% recombination for all the markers except *D5S404* and *CSF1R*.

Previous family and twin studies have provided strong evidence for the genetic determination of total serum IgE concentrations, whereas recent cellular immunologic studies have emphasized a critical role for IL-4 in regulating the differentiation of naïve T cells into T_H2 cells, promoting immunoglobulin switching to IgE and upregulating IgE biosynthesis. Our study suggests an important bridge between these findings. We propose that one or more polymorphisms in a gene or genes within human chromosome 5q31.1 are responsible for the differential regulation of overall IgE production. The most likely explanation is that one or more functional polymorphisms exist in a coding region or, more probably, a regulatory region of the *IL4* gene. However, numerous genes map within 5q31.1, including several other candidates, notably *IL13* and *IL5*, which might influence IgE production. Another possible candidate is *IRF1*, whose gene product up-regulates interferon α (*24*) which, in turn, can down-regulate IgE production (*5*). Among candidates that map distal to 5q31.1 are *IL12B*, which encodes the β chain of IL-12, and *ADRB2*, which encodes the β_2-adrenergic receptor. Interleukin-12 is a known down-regulator of T_H2 cells and of IgE production (*6*, *25*). Because the β_2-adrenergic receptor has been implicated in asthma and other atopic diseases (*26*), possible functional polymorphisms of this receptor could indirectly influence total IgE concentrations. However, these two genes do not appear to play a role in the differential production of IgE, because the closest markers, *D5S210* and *CSF1R* (Fig. 1), showed no significant associations with log[total IgE] in the sib-pair analyses.

Recent studies have revealed a complex array of positive and negative regulatory elements in the 5′ upstream regions around 300 base pairs of the mouse and human *IL4* genes (*27*). In addition, there is evidence for a mast cell–specific enhancer within the second intron of mouse *IL4* (*28*). The cytokine IL-4 is required to initiate the differentiation of naïve T cells into T_H2 cells (*6*, *8*), but early in development, there are no T_H2 cells present to produce the necessary IL-4. Basophils and possibly mast cells may fulfill this primary role (*3*) as well as contribute to this process throughout

life. It seems likely that a generalized up-regulation of IL-4 could induce B cells (that are precommitted to make IgG Ab to a broad array of Ags) to switch to IgE. Under normal physiology, such polyclonal up-regulation of IgE would not be specific for common environmental allergens and would not be detected by the multiallergen assay.

Our observations are relevant in understanding the genetic basis of asthma (*29*), where there is ample evidence for basophil and mast cell activation associated with enhanced cellular releasability (*30*). Also, associations have been observed in population studies between total serum IgE concentrations and asthma and bronchial hyperresponsiveness (*31, 32*). The log odds ratio for asthma was linearly related to log[total IgE] after adjusting for skin test reactivity, age, sex, and smoking (*31*). Also, asthma prevalence was related to log[total IgE] even in nonatopic subjects, who were skin test–negative to common aeroallergens (*31*). These findings provide the basis for further exploration of the relations between IgE phenotypes, asthma, and the functional polymorphism (or polymorphisms) of *IL4* and other candidate IgE genes in chromosome 5q31.1. Subsequent to our studies, Borish *et al.* (*33*) found further evidence to support our findings.

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17. A chemiluminescence, solid-phase multiallergen immunoassay (Magic Lite, ALK, Copenhagen-CIBA-Corning, Medfield, MA) was used to measure IgE Ab concentrations. The solid phase comprised standardized quality (SQ) extracts of the following 20 common aeroallergens: grass pollens (*Cynodon dactylon, Lolium perenne*, and *Phleum pratense*), tree pollens (*Betula verrucosa, Quercus alba, Olea europeae*, and *Cryptomeria japonica*), weed pollens (*Ambrosia artemisiifolia, Artemisia vulgaris, Plantago lanceolata*, and *Parietaria officinalis*), house-dust mites (*D. pteronyssinus* and *D. farinae*), animal danders (cat, dog, and horse), cockroach (*Blattella germanica*), and molds (*Alternaria alternata, Cladosporium herbarum*, and *Aspergillus fumigatus*). The 20 extracts (each containing 20 or more distinct allergens) were coupled separately to activated, paramagnetic particles, and the immunosorbents were standardized against controls. The immunosorbents were then blended, and the mixture was further standardized. Serum or plasma samples were allowed to incubate with the multiallergen particles and washed, and then with monoclonal Ab to human IgE labeled with acridinium ester. The particles were washed and transferred to an autoanalyzer, and the measured relative light units (RLUs) were compared with the RLUs of a standard serum pool of known relative Ab content. Two further similar immunoassays were performed with solid-phase SQ standardized extracts of the house-dust mites, *D. pteronyssinus* (*Der p*) and *D. farinae* (*Der f*). The IgE Ab concentrations were expressed in terms of arbitrary units per milliliter that were internally consistent for the different IgE Abs. The lower limits of detectability were similar for the multiallergen Ab and for each of the mite Abs. Assays were performed in duplicate and values differing by ≥10% were repeated until the coefficient of variation was within 10%. All subjects for whom IgE Abs were not detectable were assigned the lowest limit of detectability.
18. DNA microsatellite analysis was performed essentially according to J. L. Weber and P. E. May [*Am. J. Hum. Genet.* **44**, 388 (1989)] with B cell lines or lysed white blood cells (WBCs) separated by the Ficoll-Hypaque density gradient or phytohemagglutinin techniques [E. Ehrlich-Kautzky, N. Shinomiya, D. G. Marsh, *Biotechniques* **10**, 39 (1991)]. The following markers and polymerase chain reaction (PCR) primers were used (annealing temperatures in parentheses): *D5S404*, CTGGAGATGTAATGCTGTGC* and GATCACCACATTCCACCTAAT (55°C); *IL4-R1* (second intron), TGCACCTGGGCAACAGTTTA and GTTGGATGGACTTGGAGATT* (59°C); *IRF1* (seventh intron), TCATCCTCATCTGTTGTAGC and ATGGCAGATAGGTCCACCGG* (51°C); *IL9* (fourth intron), AGGCTTTCTCTAATGCAGAG* and GGTGGTTGACCTCAAATTGG (55°C); *D5S393*, TTCTACCTGNCCTTTCCTCT* and CATTCCTCATTCCTCATTCC (55°C); *D5S399*, GAGTGTATCAGTCAGGGTGC* and GGCCTCAACTTCATAATCAA (55°C); *D5S210*, ATGCAGAATCTACAAGGACC* and CTTTAACATCCTTTAACAGC (55°C); and *CSF1R* (second intron), TGTGTCCAGCCTTAGTGTGCA* and TCATCACTTCCAGAATGTGC (55°C). Seven additional highly polymorphic markers in chromosomes 1, 9, 11, and 12 were analyzed, and paternity was verified in all families. The primer sequences were obtained from various genome sequence databases (*34*), except that the optimized *IL4-R1* primer sequences were designed in our laboratory. Sequences were verified with GenBank and original sources. One primer of each pair was end-labeled (*) with [γ-^{35}S]- or [γ-^{32}P]adenosine triphosphate with T4 kinase. Each 10-μl PCR (polymerase chain reaction) mixture contained ≈300 to 1000 lysed WBC equivalents, 1.3 pmol of each PCR primer, 200 μM of each of the four deoxynucleotide triphosphates, 1.5 mM $MgCl_2$, 0.25 U of Taq polymerase (Promega) in tris buffer at pH 9.0, and the mixture was overlayed with 20 μl of mineral oil. The samples were heated for 5 min at 94°C, and PCRs were carried out (Hybaid thermocycler) with 40 cycles of denaturation (45 s, 94°C), which was followed by primer-annealing (45 s at the temperatures indicated) and extension (45 s, 72°C), and then a final extension for 10 min at 72°C. Electrophoresis was performed in urea-formamide acrylamide gels at 60 W for 2 to 4 hours. The gels were dried and exposed to Kodak XAR-5 film.
19. We also analyzed marker *IL4-R1* by agarose gel electrophoresis in 4.5% gels (GTG agarose, NuSieve) without radiolabeling, using primers described (*18*) and the primers of R. Mout, R. Willemze, and J. E. Landegent [*Nucleic Acids Res.* **19**, 3763 (1991)]. For the analysis of the *IL4-R1* alleles we utilized data from both acrylamide and agarose gel electrophoresis. *IL4-R2*, a dimorphic minisatellite also described by R. Mout *et al.*, was studied by agarose gel electrophoresis but was of little value (heterozygosity = 0.09).
20. Total IgE concentrations were measured in duplicate on serum or plasma samples from all 170 subjects by immunoassay (IMx Assay, Abbott Labs). Total IgE concentrations are expressed in nanograms per milliliter, relative to a set of standards calibrated against the International Union of Immunological Societies (IUIS) standard, taking 1 IU = 2.42 ng. The sensitivity of the assay was 0.5 ng/ml; values <1.0 ng/ml were set at 1.0 ng/ml for the subsequent statistical analyses. Values differing by ≥10% were repeated until the coefficient of variation was within 10%, except for total IgE concentrations <10 ng/ml, where the coefficient of variation was within 20%.
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22. Factors contributing to this variability in atopic, IgE Ab$^+$ subjects included (i) differences in the allergenic exposure within different microenvironments in each household, such as differential exposure to mite allergens in different beds [R. P. Young *et al.*, *Clin. Exp. Allergy* **22**, 205 (1992)]; (ii) longitudinal differences in seasonal allergen exposure; (iii) the different *Ir* genes possessed by different sibs; and (iv) differences in the periferal T cell repertoire. The effects of the environment and polygenes are likely to be more uniform within than between families. Hence, especially when one removes the "noisy" IgE Ab$^+$ subjects from consideration, the underlying genetic effect on total IgE should be more clearly evident in the sib-pair analyses.
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37. White blood cells from the family members were lysed and the DNA denatured by heating in aqueous suspension for 30 min at 95°C. Eight polymorphic DNA microsatellite markers (bold font, larger size in Fig. 1) were amplified by PCR and analyzed by electrophoresis in denaturing acrylamide gels (*18*). *IL4-R1*, a complex $(TG)_n$-$(TA)_m$ repeat in intron 2 of *IL4* (*35*) was also analyzed by agarose gel electrophoresis (*19*). The length for the shortest, most common allele of *IL4-R1* corresponds to that reported (*35*).
38. We wish to thank the Amish families for their willingness to participate in this study, W. Bias for providing cell and plasma samples from certain Amish families, R. Hamilton for help with the total IgE analyses, J. York-Blasser, RN, and S. Beiler for fieldwork, C. Stewart and G. Hansen for technical assistance, S.-K. Huang and B. Catipovic for discussions, and CIBA-Corning for provision of equipment. This research was supported by NIH grant AI20059. The program package S.A.G.E. is supported by USPHS resource grant 1 P41 RR03655 from the Division of Research Resources.

23 November 1993; accepted 22 April 1994

TECHNICAL COMMENTS

CD26 Antigen and HIV Fusion?

The human CD4 molecule generally must be expressed on a human cell type in order to support the membrane fusion reactions involved in human immunodeficiency virus type 1 (HIV-1) infection and syncytium formation (*1–7*). Studies of cell fusion mediated by recombinant CD4 and HIV-1 envelope glycoprotein (env) indicate that this species restriction is unidirectional: CD4 must be present on a human cell type, whereas env functions comparably when expressed on human or nonhuman cells (*2*). Furthermore, the fusion defect in CD4-expressing nonhuman cells can be overcome by forming stable (*5*) or transient (*6, 7*) hybrids with human cells. These findings suggest the essential involvement of a human-specific accessory component in the $CD4^+$ cell rather than the presence of a fusion inhibitor in the nonhuman cells or an inherent fusion incompatibility between human and nonhuman cell types. C. Callebaut *et al.* recently proposed that the human CD26 antigen, also known as dipeptidyl peptidase IV (DPP IV), serves as an essential cofactor for HIV entry into $CD4^+$ cells (*8*). This report prompted us to analyze the role of CD26 in HIV-1 env/CD4-mediated cell fusion. A preliminary summary of our findings has been presented (*9*).

One of our experimental approaches was based on the report by Callebaut *et al.* that transient co-expression of human CD4 and CD26 rendered murine NIH 3T3 cells permissive for HIV infection (*8*). We used a well-characterized expression system based on vaccinia vectors to study directly fusion between cells expressing recombinant HIV-1 env and cell types of several species expressing recombinant human CD4; the objective was to test whether co-expression of human CD26 could overcome the fusion defect in nonhuman cells that express CD4. For sensitive quantitative analysis, we used a newly developed assay (*10*) in which the cytoplasm of one cell population contains a transfected plasmid with the *Escherichia coli LacZ* gene linked to a T7 promoter and the cytoplasm of other cell population contains vaccinia-encoded bacteriophage T7 RNA polymerase. Cell fusion results in activation of the reporter gene selectively in the cytoplasm of the fused cells; β-galactosidase activity is quantitated in detergent cell lysates with a colorimetric assay. We also scored cell fusion using a standard assay of syncytia formation (*7, 11*). We used vaccinia vectors to induce the expression of T7 RNA polymerase, with or without CD4, in HeLa (human), NIH 3T3 (murine), and BS-C-1 (simian), cells (Fig. 1); each cell type was also transfected with either a control plasmid or a plasmid containing the CD26 complementary DNA (cDNA) linked to a strong vaccinia promoter. Flow cytometry analysis (Fig. 1A) indicated efficient expression of vaccinia-encoded CD4 on the surface of all three cell types. When CD26 cDNA was included (Fig. 1A, right panels), cell surface CD26 was readily detected in each case. The amounts of vaccinia-encoded CD26 produced in the murine and simian cells were comparable to the amounts present in the human cells without or with augmentation by the vaccinia expression system. Furthermore, separate analyses of intact cells and cell lysates showed that vaccinia-mediated CD26 expression greatly elevated DPP IV enzymatic activity, thereby confirming the functionality of the expressed protein (not shown).

To analyze cell fusion, we mixed the cell populations described above with separate populations of cells expressing vaccinia-encoded HIV-1 env and also transfected with a plasmid containing the *LacZ* gene linked to the T7 promoter. We examined functional wild-type envs from two distinct HIV-1 isolates (IIIB and SF2); as a negative control, we used a mutant uncleavable env (Unc-IIIB, derived from IIIB) that had been rendered fusion-incompetent by deletion of the normal gp120/gp41 cleavage site (*12*). After cell mixing and incubation, the detergent NP-40 was added to one set of samples and the amounts of β-galactosidase were measured (Fig. 1B). A second set of samples was analyzed microscopically for syncytia formation (Fig. 1C). When HeLa cells (Fig. 1B, top panel) expressing CD4 were mixed with cells expressing active envs from either the IIIB or the SF2 isolates, large amounts of β-galactosidase were produced. We observed only low background amounts in the negative controls when the mutant uncleavable env was used or when CD4 was omitted from the T7 RNA-polymerase–containing HeLa cells, or both. These results demonstrate that the β-galactosidase signals with the wild-type envs represented env/CD4-mediated cell fusion. Expression of vaccinia-encoded CD26 on the CD4-expressing HeLa cells had no significant effects on fusion. When we examined the murine (Fig. 1B, center panel) or simian cells (Fig. 1B, bottom panel), expression of CD4 yielded only background amounts of β-galactosidase, independent of the fusogenic nature of env on