Novel loci controlling lymphocyte proliferative response to cytokines and their clustering with loci controlling autoimmune reactions, macrophage function and lung tumor susceptibility

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Novel genotyping and statistical tools have led to mapping of numerous QTL loci for multigenic traits that previously could not be detected. The relationships of these QTL families to other QTL families and the functional specialization of their members can now be studied. We have mapped a number of loci controlling activation of T lymphocytes by mitogens and cytokines and their capacity to produce cytokines. In (O20xOcB-9)F2 hybrids, we mapped 3 novel loci controlling proliferative T-cell response to cytokines IL-2 and IL-4 (Cinda3) or IL-4 only (Cinda4 and Cinda5). OcB-9 allele at Cinda3 controls a higher response than the O20 allele to both IL-2 and IL-4, and OcB-9 alleles of Cinda4 and Cinda5 control higher response to IL-4. These novel Cinda loci and the previously mapped Cinda1 locus seem to be located in genomic regions together with other QTL families: macrophage function loci Maril1 and Maril2, proteoglycan-induced arthritis loci Pgia4, Pgia7 and Pgia12 and lung tumor susceptibility loci Sluc1, Sluc4, Sluc6 and Sluc20. The possible relevance of these QTL associations in several different sites of the genome for the immune response, inflammation and tumorigenesis has to be elucidated.

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Key words: modifiers; lymphocyte activation; interleukin 2; interleukin 4; lung cancer susceptibility

T lymphocytes with their multiple regulatory and effector functions are one of the cornerstones of vertebrate immune systems. They participate in localization and removal of virally infected and neoplastic cells, collaborate in stimulating antibody production and macrophage activation. Profound defects in T-lymphocyte functions may cause increased susceptibility to viral and bacterial infections and various autoimmune syndromes. Identification of genes underlying genetic variation of T-lymphocyte functions can contribute to the understanding of the role of T lymphocytes in disease syndromes and in normal variation in susceptibility to autoimmune diseases and infections. In previous studies, we described a number of genetic loci controlling various T-lymphocyte functions, including proliferation and production of cytokines after mitogen stimulation, as well as response to stimulation by cytokine IL-2.1–3 Interleukin 2 belongs, together with IL-4, IL-7, IL-15 and IL-21, to the common cytokine-receptor γ chain family cytokines. The receptors for these cytokines share a common γ chain (IL-2Rγ, IL-4Rγ, IL-7Rγ, IL-15γ, IL-21Rγ) and act as T-cell growth and survival factors.4 They have been implicated in an array of biologic effects on several cell lineages.4–6 IL-2 and IL-4 have been shown to stimulate natural killer cells and T lymphocytes,8 respectively, to destruct tumor metastases in lung. Cell activation via IL-2R and IL-4R might be influenced by many factors such as size of the responding cell subpopulations, presence of costimulatory molecules on the cell surface,5 autocrine synthesis of cytokines, ligand receptor ratio, which might determine involvement of different signal transmission pathways.6,11

To localize the gene(s) that influences the intensity of the response to IL-2 and IL-4, we have used the recombinant congenic (RC) strains, which were developed for genetic analysis of multigenically controlled biologic processes.12 Each RC strain contains a different, random set of approximately 12.5% genes of the common donor strain and approximately 87.5% genes of the common background strain. The RC strains may be especially useful in analysis of relationships of different components of complex traits, such as susceptibility to infection.13,14 In the present study, we analyzed response to IL-2 and IL-4 in O20-c-B10.O20/Dem (OcB/Dem) series. In the pilot experiment, we tested response of splenocytes of parental strains O20/A (O20) and B10.O20/Dem and OcB/Dem strains. The RC strain OcB-9/Dem (OcB-9), which has 87.5% of genes from the strain O20 and 12.5% of genes from the strain B10.O20,15 exhibited significant differences in response to IL-2 and IL-4 from the background parental strain O20. In order to map the loci responsible for this difference, we analyzed IL-2 and IL-4 response of F2 hybrids between this RC strain and the parental strain O20. This revealed 3 novel Cinda loci.

Unexpectedly, these loci turned out to be associated with loci controlling macrophage function, proteoglycan-induced rheumatoid arthritis and susceptibility to lung tumors, but also intestinal tumors and lymphomas. The possible significance of this colocalization of combinations of genes of the same classes in several positions of the genome is discussed.

Material and methods

Mice

Mice, both male and female, of strains O20, B10.O20, OcB-9 and F2 hybrids between OcB-9 and O20 came from P. Demant’s breeding colony. The genetic composition of strain OcB-9 has been described in detail by Stassen et al.15 The strains O20 and OcB-9 were in at least generation 100 and 31, respectively, of inbreeding and therefore highly homozygous. Age of mice was 8–20 weeks (mean and median, 13 weeks).

Interleukins

Human recombinant interleukin 2 (hIL-2; sp. act. 18 × 106 IU/mg) was obtained from EuroCetus Benelux [Amsterdam, The Netherlands; IE (Cetus) = 2 Roche units = 6 IU]. Myeloma cell line X63Ag8-653 transformed by mouse IL-2 or IL-4 cDNA,16 a gift from Dr. F. Melchers, Basel Institute for Immunology, in Basel, Switzerland, served as a source of mouse IL-2 (mIL-2) and IL-4 (mIL-4), respectively.

Lymphocyte proliferation assay

Spleen cells (105 cells per well) from parental strains and F2 hybrids were incubated in 96-well tissue culture plates in complete...
To obtain whole genome significance values (corrected by Kruglyak19) using the formula 
the NCSS package. All p-ratio). The Spearman correlation coefficients were computed by 
the estimated distance from a Sluc observed and in the response to the highest doses of hIL-2 and mIL-4 than the background parental strain O20. The RC strain OcB-9 exhibited higher proliferative response to 
15.62, 31.25, 125 and 500 ng/ml mIL-2; and 0.47, 0.94, 3.75 and 15 ng/ml mIL-4 as described elsewhere.17 [3H]-thymidine (0.5 μCi/well; Institute for Research, Development and Utilization of Radioisotopes, Prague, Czech Republic) was added to the cultures for the last 6 hr of the 72-hr incubation period. Stimulation indexes were calculated as follows: 
stimulation index = \frac{cpm \text{ cytokine induced proliferation}}{cpm \text{ cells in medium only}}.

Each value represents the mean of 3 independent measurements.

Genotyping of simple sequence length polymorphism (SSLP) by PCR

DNA was isolated from tails using a standard proteinase procedure. The strain OcB-9 differs from O20 at B10.O20-derived segments on 10 chromosomes.15 These differential segments were typed in the F2 hybrid mice using 26 microsatellite markers: D2Mit7, D2Mit56, D4Mit72, D4Mit54, D4Mit126, D6Mit31, D6Mit52, D7Nds2, D7Mit39, D7Mit12, D7Nds4, D8Mit3, D8Mit65, D10Mit12, D10Mit122, D11Mit15, D16Mit130, D18Mit14, D18Mit35, D19Mit61, D19Mit63, D19Mit9, D19Mit3 and D19Mit33. The maximum distance between any 2 markers in the chromosomal segments derived from the B10.O20 strain or from the nearest O20-derived markers was 17 cm. PCR genotyping was performed as described elsewhere.18

Statistical analysis

The role of genetic factors in proliferative response was examined by analysis of variance (ANOVA; NCSS, Kayville, UT). Marker, gender and age were fixed factors and the experiment was considered a random parameter. Both main effects and interactions between factors were analyzed. The proliferative response was expressed either as stimulation index or the counts of nonstimulated suspensions were subtracted from the counts of suspensions stimulated with IL-2 or IL-4. Both calculations gave similar results. In order to obtain normal distribution of the analyzed responses as required for ANOVA, the obtained values were transformed as shown in the legends of Tables I–III. Markers and interactions with p < 0.05 were combined in a single comparison. To obtain whole genome significance values (corrected p), the observed p-values (p scan) were corrected according to Lander and Kruglyak19 using the formula \alpha * = \min 1, \alpha (G) + 2G \times \beta (T), \alpha scan, with G = 2 Morgan (the length of the segregating part of the genome: 12.5% of 16 M), C = 10 (number of chromosomes segregating in this cross), \beta scan = 1.5 for F2 hybrids and \beta (T) = the observed statistics (F ratio). The Spearman correlation coefficients were computed by the NCSS package. All p-values mentioned in the text are corrected p-values.

The probability that all detected Cinda loci were located within the estimated distance from a Sluc locus was evaluated by binomial distribution considering that 7 Sluc loci were detected in strain OcB-9 in the 230 cM of its genome that comprise segments of donor strain origin.20–22

Results

Strain differences in proliferative responses to IL-2 and IL-4

We analyzed the responses of these strain differences by examining the response to 125, 250, 500, 1,000 and 2,000 IE/ml hIL-2; 15.62, 31.25, 125 and 500 ng/ml mIL-2; and 0.47, 0.94, 3.75 and 15 ng/ml mIL-4. Splenocytes of parental strains B10.O20 and O20 differed in response to IL-2 and IL-4 (Fig. 1). The donor strain B10.O20 is a higher responder to both hIL-2 and mIL-2 than the background parental strain O20, while their response to IL-4 is opposite; O20 is a higher responder to this cytokine than B10.O20. The RC strain OcB-9 exhibited higher proliferative response to hIL-2, mIL-2 and mIL-4 than the background parental strain O20 and in the response to the highest doses of hIL-2 and mIL-4 differed from both parental strains O20 and B10.O20. Response of (OcB-9 × O20)F2 hybrids to hIL-2 and mIL-2 is highly correlated. A high correlation was observed between lower concentrations of hIL-2 (125 IE/ml) and all concentrations of mIL-2 tested (R ranging from 0.70 to 0.78; all p-values < 0.00001). The correlation coefficients of response to 2,000 IE/ml hIL-2 with mIL-2 responses ranged from 0.48 to 0.57 (all p-values < 0.00001). These correlations suggested that response to hIL-2 and mIL-2 are likely controlled by similar or identical sets of genes. Correlation was also observed between response to mIL-4 and hIL-2 (R ranging from 0.48 to 0.68; all p-values < 0.00001). Also, high correlation was observed between response to high concentration of IL-4 (15 ng/ml) and all concentrations of mIL-2 tested (R ranging from 0.63 to 0.69; all p-values < 0.00001), and lower but highly significant correlation response to lower concentration of IL-4 (0.47 and 0.94 ng/ml) and high concentration of mIL-2 (500 ng/ml, R = 0.31 and 0.28; p < 0.00001). These correlations strongly suggest that several loci controlling response to IL-2 and IL-4 in this cross are identical. In these and subsequent linkage experiments, the same evidence for linkage and type of regulation was observed when the counts of nonstimulated suspensions were subtracted from the counts of suspensions with interleukins (data not shown).
New locus Cinda3, controlling proliferative response to hIL-2, mIL-2 and IL-4

In the (OcB-9 × O20) F2 hybrids, the response to doses of 125, 250, 500 and 1,000 IE/ml hIL-2 was linked to the marker D4Mit126 (corrected p = 0.0327, 0.00568, 0.00215 and 0.00369, respectively), the highest response being observed in heterozygotes and lowest in O20 homozygotes (Table I). These data indicate the presence of a locus, Cinda3 (cytokine-induced activation 3), in this region. Females exhibited higher proliferative response in lower- and intermediate-dose region (p < 10^−7, 0.000034 and 0.000191 for 125, 250 and 500 IE/ml, respectively). To the marker D4Mit126 is linked also the response to mIL-2 at 125 ng/ml of mIL-2 (corrected p = 0.0030). Similarly with hIL-2, the highest response was in heterozygotes and lowest in O20 homozygotes (Table II). Females are better responders than males (p < 0.0023). Finally, the response to IL-4 at 0.94 ng/ml is also linked to D4Mit126 (corrected p = 0.00049), heterozygotes being again the highest and O20/A homozygotes the lowest responders and females responded better than males (p = 0.01; Table III). Apparently, Cinda3 controls the responses to both IL-2 and IL-4. As the marker D4Mit54, located 5 cm centromerically, appears to be also linked to IL-2 responses (p = 0.06), the Cinda3 maps most likely between these 2 loci. Inside the D4Mit54–D4Mit126 interval, 4 cm from D4Mit126, is D4Mit158, which defines the lung tumor susceptibility locus Sluc622 (Fig. 2).

Two loci, Cinda4 and Cinda5, controlling response to IL-4 but not IL-2

In addition to Cinda3 on chromosome 4, 2 novel loci (Table III) influenced response to IL-4. Cinda4 controls response to low concentration of IL-4 (0.94 ng/ml) and is linked to the marker D19Mit9 on chromosome 19 (corrected p = 0.0295), but not to the more centromeric D19Mit63 or more telomeric D19Mit53 (data not shown). The highest response is observed in homozygotes for the B10.O2O allele. The Sluc1 locus has been mapped to D19Mit9 as well.20 In view of comapping of the 2 loci to the same marker, they either are located in the same position or their distance is small (Fig. 2).

The response to the high concentration (15 ng/ml) of mIL-4 maps to the markers D8Mit3 and D8Mit65 on chromosome 8 (corrected p = 0.0317 and 0.00207, respectively). Similarly to Cinda4, the B10.O2O allele is dominant and determines highest proliferative response (Table III). Females exhibited higher response than males (p = 0.028 and 0.022 for D8Mit65 and D8Mit3, respectively). These data indicate the presence of a novel locus, Cinda5, located with the highest probability in the 12 cm segment between D8Mit3 and D8Mit65, although the presence of more than one Cinda5 cannot be excluded. The Sluc20 locus maps also to the marker D8Mit3. The extent of the donor strain segments of chromosome 8 in OcB-9 (Fig. 2) indicates that in the extremely unlikely case that Sluc20 and Cinda5 are located at the extreme ends of the 2 donor strain segments on chromosome 8, they are 22 cm apart. However, a considerably shorter distance is much more probable.

Although the precise locations of the Cinda and Sluc loci paired on chromosomes 4, 8 and 19 are not yet known, the mapping data indicate that they are close together and possibly identical. Cinda3 maps 4 cm from Sluc6. The position of Cinda4 is less precisely mapped but one of the markers to which it localizes is identical to that of Sluc20. Cinda5 maps to the same marker as Sluc1. This is compatible with the location of each Cinda-Sluc pair being within a 3 cm region. The probability that the Cinda and Sluc loci are distributed in the genome randomly and that such comapping occurred by chance is only 0.0462 (binomial distribution), indicating that the Cinda and Sluc loci are not randomly distributed but located close to each other. This hypothesis is further strengthened by the colocalization of Cinda1 and Sluc4 observed in independent experiments in other strains.3,20

Discussion

The RC strain OcB-9 is a higher responder than the parental strain O20 to hIL-2, mIL-2 and mIL-4 and also a higher responder to highest doses of hIL-2 and mIL-4 than the second parental strain B10.O2O (Fig. 1), indicating that specific allelic combinations of several genes interact to generate its high responses. As it contains only 12.5% of genes from B10.O2O, it probably does not contain the genes responsible for the low response of B10.O2O lymphocytes to IL-4. We have defined 3 novel loci that control proliferative response to IL-2 and IL-4. Cinda3 influences response to hIL-2, high concentrations of mIL-2 and low concentrations of mIL-4. Cinda4 and Cinda5 determine response to low and high IL-4 concentrations, respectively (Tables I–III). None of the 3 regions near Cinda loci contains IL-2 or IL-4 genes or their receptors. Cinda3, which controls the proliferative response to 125–1,000 IE/ml hIL-2, 125 ng/ml mIL-2 and 0.94 ng/ml IL-4, is linked to the marker D4Mit126 on the distal part of chromosome 4. The observation that response to hIL-2, mIL-2 and IL-4 is controlled by the same locus Cinda3 and that the control of response is similar, the highest response being in all cases observed in heterozygotes and lowest in O20 homozygotes, might be related to the fact that signal from both IL-2R and IL-4R is transmitted by a common γ chain and that both signaling pathways share several components.4–6 Alternatively, closely linked genes in this region determine separately response to IL-2 and IL-4. The question whether the response to IL-2 and IL-4 is influenced by a single gene or by several linked genes and whether any of the genes listed above each genotype, O and B indicate the presence of O20 and B10.O2O allele, respectively. p-values that were significant after correction for genomewide significance (corrected p < 0.05) are underlined.

### Table 1 - Linkages of Proliferative Response to hIL-2 in (OcB-9 × O20)F2 Hybrids

<table>
<thead>
<tr>
<th>Genotype</th>
<th>p</th>
<th>Corrected p</th>
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<tbody>
<tr>
<td><strong>OO</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 IE/ml</td>
<td>1.39 ± 0.04</td>
<td>1.65 ± 0.03</td>
</tr>
<tr>
<td>500 IE/ml</td>
<td>2.05 ± 0.08</td>
<td>2.36 ± 0.03</td>
</tr>
<tr>
<td>250 IE/ml</td>
<td>1.62 ± 0.04</td>
<td>1.91 ± 0.03</td>
</tr>
<tr>
<td>1,000 IE/ml</td>
<td>2.62 ± 0.05</td>
<td>2.96 ± 0.03</td>
</tr>
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</table>

Marker D4Mit126. Only linkages significant at whole genome level are given. The numbers give the natural logarithm of the stimulation index of spleen cell stimulated by 125, 250, 500 and 1,000 IE/ml hIL-2; n indicates number of mice. These values, using the same transformation as in F2 hybrids, in the parental strains O2O (n = 29) and OcB-9 (n = 24) were 1.13 ± 0.06 and 1.80 ± 0.07 (p < 0.0000002); 1.40 ± 0.06 and 2.05 ± 0.07 (p < 0.00000003); 1.73 ± 0.06 and 2.32 ± 0.07 (p < 0.0000004); 2.09 ± 0.06 and 2.60 ± 0.07 (p < 0.00000038) when 125, 250, 500 and 1,000 IE/ml were used, respectively. The numbers in bold give the average nontransformed stimulation index for each genotype. O and B indicate the presence of O20 and B10.O2O allele, respectively.
the same locus. This is likely due to the ±60% homology between human and mouse IL-2.24 These findings are in agreement with the characteristics of these responses in F2 hybrids between CcS-4 and BALB/c.3

Tlsr3 (thymic lymphoma suppressor region 3) and Smml (stathmin 1) that influence both lymphocyte growth and cancer development as well as another lung tumor susceptibility locus, Sluc1, are localized in the vicinity of Cinda3, but more centromerically (http://www.informatics.jax.org). As discussed above, much closer to Cinda3 are several other loci affecting cancer development: Sluc6 (susceptibility to lung cancer),22 ssc1 (susceptibility to small intestinal cancer),25 and Momi1 (modifier of Min-1),26 which is involved in intestinal neuroplasms and was identified as PlagZg2a (phospholipase A2, group II A),26,27 an enzyme with pleiotropic functions that has been implicated in many different biologic responses, including inflammation, cell signaling and antimicrobial activity.26,27

With the exception of a short centromeric segment, the chromosome 19 in the strain Oe-B9 is of B10.O20 origin. Cinda4 that influences response to low concentrations of IL-4 is in its telomeric part. The lung tumor susceptibility gene, Sluc1, maps to the same marker as Cinda4.26 Only 2 and 4 cM more telomeraically are genes Msx1 (max interacting protein 1) and Csf2ra (colony-stimulating factor 2 receptor, alpha, low affinity), which influence lymphocyte proliferation (http://www.informatics.jax.org), but no influence of their polymorphism on IL-4-stimulated cell growth has been reported.

Cinda5 is marked by D8Mit3 and D8Mit65 in the second proximal B10.O20-derived segment (Fig. 2) chromosome 8 in close vicinity of Sluc20 that also maps to D8Mit3.24 No obvious candidate genes are known in these regions.

Our data indicate complex control of cytokine-induced proliferation. We have found that response to 125–1,000 IE/ml H–L2 is controlled by one locus, whereas response to different concentrations of IL-4 is controlled by different loci (Tables I–III). It is possible that genes that operate in a wide range of concentrations (e.g., Cinda3) might participate in some essential pathways; those that control response at only some concentrations (e.g., Cinda4 and Cinda5) might participate in pathways that are preferentially activated at certain conditions. Because an imbalance in cytokine expression was described in many pathologic conditions, cytokines are being used in immunotherapy. Identification of relevant genes, such as Cinda in the mouse, would make it possible to test the role of their human homologues in the responsiveness to cytokine immunotherapy.

We have found that all 3 newly described Cinda loci map in the near vicinity of loci controlling cancer susceptibility. Cinda3 is near to Sluc6, ssc1, Plag2g2a, Tlsr3 and Smml; Cinda4 maps to the same position as Sluc1; Cinda5 is near to Sluc20. Interestingly, the locus Cinda1 previously detected on chromosome 11q near the markers D11Nds19, D11Nds9 and D11Mit26 in the CcS-4 RC strain is located close to Sluc4.28 It was not detected in the present cross, possibly for stochastic reasons, or because it is detectable only on certain genetic backgrounds. The observed colocalization of Cinda and Sluc loci is statistically significantly different from their independent distribution. This can indicate that the differences of tumor susceptibility attributed to the Sluc loci are actually due to differences in lymphocyte-cytokine interactions and that Sluc loci merely reflect phenotypic manifestations of the Cinda genes at tumor susceptibility level. Alternatively, the lymphocyte activating genes and the Sluc genes occur in several closely linked complexes in the genome. It was hypothesized22 that some tumor susceptibility genes may operate through regulation of inflammation. Our data indicate that some genes affecting lymphocyte activation may be involved in tumor susceptibility as well. The involvement of immune functions in Sluc effects is supported by the observation that loci controlling susceptibility to proteoglycan-induced arthritis mapped independently from our experiments apparently map within 1 cM of 3 out of 4 Sluc loci implicated in the present study generating clusters: Cinda4-Sluc1, Ptgj2A; Cinda1-Sluc4, Ptgj7; and Cinda5-Sluc20, Ptgj4.30 However, the possible basis of relationship between genetic control of cytokine responsiveness and tumor susceptibility may also be due to common signaling pathways in lymphocytes and in tumor cells.

Non small cell lung carcinoma cells and normal pneumocytes express functional IL–4 receptor, including the common γ chain of

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**TABLE II – LINKAGE OF PROLIFERATIVE RESPONSE TO mIL-2 IN (Oe-B-9 × Oe-B)F2 HYBRIDS**

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<th>Genotype</th>
<th>p</th>
<th>Corrected p</th>
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<tbody>
<tr>
<td></td>
<td>OO</td>
<td>OR</td>
<td>BB</td>
</tr>
<tr>
<td>D4Mit126</td>
<td>1.35 ± 0.05</td>
<td>1.67 ± 0.03</td>
<td>1.55 ± 0.05</td>
</tr>
<tr>
<td>3.86 (n = 55)</td>
<td>5.31 (n = 122)</td>
<td>4.71 (n = 49)</td>
<td></td>
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</tbody>
</table>

Only linkages significant at whole genome level are given. The natural logarithm of the stimulation index of spleen cells stimulated by 125 ng/ml IL-2 is controlled by different loci (Tables I–III). It is possible that genes that operate at only some concentrations (e.g., Cinda4 and Cinda5) might participate in pathways that are preferentially activated at certain conditions. Because an imbalance in cytokine expression was described in many pathologic conditions, cytokines are being used in immunotherapy. Identification of relevant genes, such as Cinda in the mouse, would make it possible to test the role of their human homologues in the responsiveness to cytokine immunotherapy.

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**TABLE III – LINKAGES OF PROLIFERATIVE RESPONSE TO mIL-4 IN (Oe-B-9 × Oe-B)F2 HYBRIDS**

<table>
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<th>Marker</th>
<th>Genotype</th>
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<tr>
<td></td>
<td>OO</td>
<td>OR</td>
<td>BB</td>
</tr>
<tr>
<td>D4Mit126</td>
<td>1.16 ± 0.04</td>
<td>1.43 ± 0.03</td>
<td>1.31 ± 0.04</td>
</tr>
<tr>
<td>3.19 (n = 54)</td>
<td>4.18 (n = 122)</td>
<td>3.71 (n = 48)</td>
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<tr>
<td>D19Mit9</td>
<td>1.39 ± 0.04</td>
<td>1.27 ± 0.02</td>
<td>1.50 ± 0.04</td>
</tr>
<tr>
<td>4.01 (n = 49)</td>
<td>3.56 (n = 134)</td>
<td>4.48 (n = 42)</td>
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<tr>
<td>D8Mit3</td>
<td>14.97 ± 0.82</td>
<td>20.3 ± 0.57</td>
<td>19.4 ± 0.84</td>
</tr>
<tr>
<td>12 (n = 56)</td>
<td>11 (n = 114)</td>
<td>(n = 53)</td>
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</tr>
<tr>
<td>D8Mit65</td>
<td>15.13 ± 0.76</td>
<td>21.4 ± 0.60</td>
<td>20.4 ± 0.73</td>
</tr>
<tr>
<td>12 (n = 60)</td>
<td>98 (n = 98)</td>
<td>65 (n = 65)</td>
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</table>

Only linkages significant at whole genome level are given. The natural logarithm was used to obtain normal distribution in 0.94 ng/ml: 15 ng/ml exhibited normal distribution without transformation; n indicates number of mice. These values, using the same transformation as in F2 hybrids, in the parental strains O20 (n = 29) and Oe-B-9 (n = 24) were 1.24 ± 0.06 and 1.41 ± 0.06 (p < 0.0000001); 12.49 ± 1.00 and 16.91 ± 1.10 (p < 0.00015) when 0.94 D4Mit126 and D19Mit9 and 15 ng/ml (D8Mit3 and D8Mit65) were used, respectively. The numbers in bold give the average nontransformed stimulation index for each genotype. O and B indicate the presence of O20 and B10.O20 allele, respectively. p-values that were significant after correction for genomewide significance (corrected p < 0.05) are underlined.
the IL-2 receptor. Genes that influence intracellular and intensity of signaling through IL-4R in lymphocytes might modify such responses in both lymphocytes and tumor cells. Thus, the association between 

\[ \text{Cinda} \] and \n
\[ \text{Sluc} \] effects need not imply functional effects of lymphocytes on lung tumor cells, but may reflect cell-autonomous functions of both tumor cells and lymphocytes. Recently, Fijneman et al. showed that 3 loci controlling activity of bone marrow macrophages, \n
\[ \text{Marif1} \] and \n
\[ \text{Marif2} \], are linked to the same \n
\[ \text{Sluc} \] loci as the \n
\[ \text{Cinda3} \] and \n
\[ \text{Cinda5} \], and \n
\[ \text{Marif3} \] locus is on the same chromosome as \n
\[ \text{Cinda4} \] and \n
\[ \text{Sluc1} \], but at some distance from them. It remains to be seen whether this finding reflects genetic effects on functional interactions between T lymphocytes and macrophages, or on homologous intracellular processes. The genetic studies of cytokine effects are complementing biochemical analysis of cytokine signaling pathways and could help to find critical points that are responsible for genetic differences in IL-2- and IL-4-induced response. They will also help elucidate the possible relationship between lymphocyte activation and cancer development.

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