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BRIEF COMMUNICATION

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A novel alloreactivity-controlling locus, *Alan1*, mapped to mouse Chromosome 17

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The strength of the allotransplantation reaction depends on the genetic disparity between donor and recipient and on the genotype of the recipient. It has been shown that some inbred strains respond to alloantigens with a considerably stronger immune response than the other strains (Stepkowski and Ito 1990; Stewart et al. 1985). We have described previously that the proliferative response to alloantigens in MLC is controlled by a set of nonlinked genes which regulate the response irrespectively of the major histocompatibility complex (MHC) genotype of stimulating cells (Holáň et al. 1996). Since the proliferative response in MLC correlates with the survival of subsequent allografts as well as with graft-versus-host reaction (Bach 1970; Häyry et al. 1972), the definition of genes controlling MLC responsiveness may improve the prediction of the transplantation outcome. To identify these genes, we are using a system of recombinant congenic strains (RCS) of mice produced and characterized by Demant and coworkers (Demant and Hart 1986; Stassen et al. 1996). In the RCS used in our experiments, random sets of approximately 12.5% genes from STS/A (STS) strain were transferred to the genetic background of BALB/cHeA (BALB/c) strain and a panel of 20 homozygous RCS of BALB/c-c-STS/Dem (CcS/Dem) series was prepared. The positions of STS segments in individual RCS were characterized by means of more than 600 microsatellite markers (Stassen et al. 1996). Using this approach we report here the identification of a novel alloreactivity-controlling locus, which we designate *Alan1 (Alloantigen response 1)* and which we mapped to mouse Chromosome (Chr) 17.

We have shown previously that the inbred strain STS ($H2^{dx}$) exhibits a higher proliferative response in MLC to stimulator cells of all 10 tested inbred mouse strains with 9 different MHC haplotypes than the strain BALB/c ($H2^{d}$) (Holáň et al. 1996). The MLC reactivity of 20 individual RC strains of the CcS/Dem series was then evaluated and significant differences in the proliferative response to alloantigens were found. The pattern of distribution of MLC reactivity in individual RCS was not influenced by MHC or non-MHC alloantigens on stimulating cells and did not correlate with the alloantigen-induced IL-2 production (Holáň et al. 1996). The high responder strain CcS-5 was then selected for further analysis. To map loci controlling alloreactivity, 91 male (CcS-5×BALB/c)F_2 hybrids were prepared and the reactivity of their spleen cells against C57BL/10Sn (B10) and DBA/1 stimulatory cells was tested in MLC, as described previously (Holáň et al. 1996). The strain CcS-5 carries segments of genetic material of STS origin on eight chromosomes (Stassen et al. 1996, and unpublished data). These segments were typed in F_2 hybrids by 16 microsatelite markers: *D5Mit112, D5Mit164* (Chr 5); *D6Mit173, D6Mit122, D6Mit10* (Chr 6); *D8Mit155, D8Mit17* (Chr 8); *D10Mit46, D10Mit25* (Chr 10); *D11Mit62* (Chr 11); *D16Mit91* (Chr 16); D17Mit19, *D17Mit30, D17Mit51, D17Mit138* (Chr 17); and *D18Mit19* (Chr 18) using the polymerase chain reaction (Dietrich et al. 1992). The maximum distance between any two markers on the chromosomal segments derived from the STS strain or from the nearest BALB/c-derived markers was 15.7 cM. To evaluate the proliferative response in MLC,

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Table 1 Linkage analysis of anti-B10 and anti-DBA/1 proliferative reactivities of (CcS-5×BALB/c)F_2 hybrids

Marker	Anti-B10 response					Anti-DBA/1 response				
	Genotype			*P-value*	Corrected *P-value*	Genotype			*P-value*	Corrected *P-value*
	CC	CS	SS			CC	CS	SS		
D17Mit19	1.19[a]±0.10 (19)	1.30±0.07 (46)	1.66±0.09 (24)	0.0026	N.S[b].	1.27±0.11 (19)	1.39±0.07 (46)	1.69±0.10 (24)	0.0154	N.S.
D17Mit30	1.22±0.11 (15)	1.17±0.06 (51)	1.75±0.09 (25)	0.00000599	0.00099	1.25±0.13 (15)	1.32±0.07 (51)	1.74±0.10 (25)	0.0014	N.S.
D17Mit51	1.20±0.12 (13)	1.24±0.06 (48)	1.70±0.08 (28)	0.00024	0.031	1.28±0.13 (13)	1.33±0.07 (48)	1.78±0.09 (28)	0.00061	0.053
D17Mit138	1.23±0.12 (15)	1.22±0.07 (46)	1.66±0.09 (29)	0.0008	N.S.	1.31±0.12 (15)	1.30±0.07 (46)	1.75±0.09 (29)	0.000918	N.S.

[a]The value shows natural logarithmic transformation of the ratio of the proliferative response of spleen cells stimulated with B10 or DBA/1 cells and the proliferation obtained with the same cell suspension incubated in medium only. These values for the parental strains BALB/c (n=4) and CcS-5 (n=5) were with B10-stimulating cells 0.83±0.18 and 1.53±0.17 (P<0.001) and 0.86±0.26 and 1.70±0.23 (P<0.03) with DBA/1 stimulating cells. Figures in parentheses show number of mice tested. S and C indicate presence of STS and BALB/c alleles, respectively.
[b]N.S. Not significant

counts expressing [^{3}H]-incorporation in alloantigen-stimulated cultures were divided by the counts obtained when the same cells were maintained in medium only. In order to obtain normal distribution of the counts required for ANOVA, we used natural logarithm of measured values. Evaluation of linkage was performed by analysis of variance (ANOVA, NCSS), using the marker, gender, and age as fixed factors and the day of experiment as a random factor. P-values were corrected for multiple testing according to Lander and Kruglyak (1995).

The proliferative response in MLC of all 91 (CcS-5×BALB/c)F2 hybrids was tested against B10 (*H2*^{b}) and DBA/1 (*H2*^{q}) allogeneic stimulatory cells. The B10 and DBA/1 strains differ from the responder mice at the entire MHC and at multiple non-MHC loci. The response to these two types of allogeneic cells (B10 and DBA/1) in F_2 hybrids was highly correlated (correlation coefficient R=0.58, P=0.0001). Subsequent statistical evaluation of the proliferative reactivity against B10 stimulatory cells revealed linkage of the response to the markers *D17Mit30* and *D17Mit51* on Chr 17 (corrected genome-wide P-values 0.00099 and 0.031, respectively). The statistical analysis of the anti-DBA/1 response also indicates linkage of the MLC reaction to marker *D17Mit51* (corrected P-value 0.053) (Table 1). The STS allele determines higher proliferative response. The ratio stimulating/control cultures in high responder strain STS is about 60% higher than in the low responder strain BALB/c. These data indicate the presence of a novel locus on Chr 17 controlling the proliferative response to alloantigens. We designated this locus *Alan1 (Alloantigen response 1)*.

Figure 1 shows the genetic composition of Chr 17 in strain CcS-5 and the position of markers used for linkage analysis. According to statistical significance of the linkage of response to the individual markers, the most probable position of locus *Alan1* is centromeric from the H2 complex. Although the corrected P-values for the marker *D17Mit138* did not exceed the significance limit, the position of locus *Alan1* within the H2 complex cannot be excluded.

So far, a number of various traits associated with the function of the immune system, both in rodents and human, have been shown to be linked to the

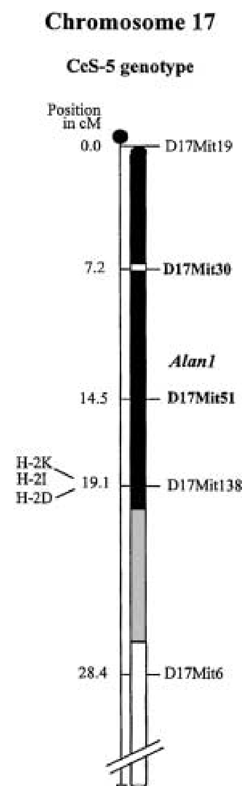

Fig. 1 Localization of *Alan1* on Chr 17. The positions of microsatelite markers used for genotyping are shown. The *dark region* indicates the most probable localization of *Alan1*. The *shaded region* indicates the possible extent of the donor strain segment of the Chr 17 in the CcS-5 strain. The markers which exhibit significant corrected P-values (P<0.05) in response to B10 and DBA/1 alloantigens are shown in *bold*

MHC (Ollier 1997; Wicker et al. 1995). A recently described locus *Tria3* (T-cell receptor induced activation 3), which controls the T-cell proliferative response to anti-CD3 stimulation, has been mapped to the vicinity of the MHC on Chr 17 (Havelková et al. 1999). We do not know whether the loci *Alan1* and *Tria3* are identical, but the patterns of proliferative responsiveness of individual RCS after stimulation with alloantigens and anti-CD3 stimulation are different. Only more detailed recombinant mapping can decide whether the locus *Alan1* is identical with a previously described genetic locus or whether it represents a novel, so far unidentified locus, or represents one or several closely linked genes.

We have already used RC strains to separate and identify genes that control T-cell proliferative responses after stimulation with IL-2 and anti-CD3 (Krulová et al. 1997; Lipoldová et al. 1995), for loci influencing production of cytokines (Kosařová et al. 1999) and for loci that control resistance to infection (Demant et al. 1996; Lipoldová et al. 2000). The results presented here extend the potential of the RCS system for the study of transplantation immunity. The identification of genes that control the immune response to alloantigens will increase our insight into the genetic basis of allotransplantation reactions and may potentially lead to improved selection of recipients for clinical transplantation. This can be tested *in vivo* by comparing survival of tissue or organ allografts in recipients bearing genes controlling a high or low responder phenotypes to alloantigens. Identification of human homologues of mouse genes controlling alloreactivity could make possible the classification of graft recipients into high and low responders. This could allow one to use a wider range of donors for transplantation in low responders who may not require precisely matched graft. On the other hand, high responders may receive the best matched grafts. Similarly, immunosuppressive treatment may be planned more precisely according to the alloreactivity phenotype of the recipient.

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