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The gene controlling the binding sites of *Dolichos biflorus* agglutinin, *Dlb-1*, is on chromosome 11 of the mouse

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Summary

A locus, *Dlb-1*, controlling the binding of the lectin from *Dolichos biflorus* to the intestinal epithelium and vascular endothelium of mice has been located on chromosome 11, 3.1 ± 1.4 centimorgans proximal to *Rex*, using recombinant inbred strains and conventional backcrosses with three-point linkage tests.

The two alleles so far discovered at this locus behave unusually. The *Dlb-1*^{a} allele causes binding to the vascular endothelium but not to the intestinal epithelium while *Dlb-1*^{b} induces the exact reciprocal binding pattern, and the heterozygote shows both patterns.

1. Introduction

Ponder & Wilkinson (1983) made a systematic study of the binding of a number of different lectins to tissue sections in different inbred strains of mice. The *Dolichos biflorus* lectin, with specificity for terminal, non-reducing *N*-acetyl galactosamine residues, was found to have a strain-specific binding pattern to intestinal mucosa and vascular endothelium. In some strains (e.g. RIII-*ro*, DDK, GRS/A, STS/A and SWR) the lectin bound to the vascular endothelium, but not the intestinal epithelium, while in most other strains tested (e.g. C57BL, DBA/2 and BALB/c) the converse was true. A total of over 30 strains have now been typed (Ponder, Festing & Wilkinson, 1985; Uiterdijk & Ponder, 1985).

Subsequent studies involving crosses between these two types of strains (Ponder, Festing & Wilkinson, 1985; Uiterdijk & Ponder, 1985) suggested that this pattern was controlled by a single locus with a codominant mode of inheritance, i.e. in the F_1 hybrid the lectin bound to both tissues. No mice have yet been found in which the binding is negative in both tissues. The locus was given the gene symbol *Dlb-1* (Dolichos Lectin Binding-1), with the *Dlb-1*^{a} allele (type strain RIII-*ro*) causing binding to the vascular endothelium, and the *Dlb-1*^{b} allele (type strain C57BL/6) binding to the intestinal epithelium.

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We now report on the chromosomal location of the gene controlling the binding of this lectin.

2. Materials and Methods

The genetic mapping was carried out simultaneously in The Netherlands and the U.K., and the data have been combined to form a single report.

Mice were typed for their *Dlb-1* phenotype using methods described by Ponder & Wilkinson (1983). The exact methods used differed between laboratories, but this is unlikely to have influenced the results. Briefly, the mice were killed, and 2–3 cm lengths of the distal small intestine were fixed in Carnoy's fixative or 10% formol saline at room temperature. These were then embedded in paraffin wax, and sectioned. Sections were de-waxed, the endogenous peroxidase was blocked using 0.1% phenylhydrazine hydrochloride and the sections were reacted with *Dolichos biflorus* lectin conjugated with horseradish peroxidase. Colour was developed using 3.3′ diaminobenzidine as substrate, and the sections were examined for staining of the intestinal epithelium and vascular endothelium. Control sections were incubated with the lectin conjugate in the presence of the inhibiting sugar or without the lectin conjugate. Haemalum was used as a counterstain. No sex differences for the *Dlb-1* phenotype were observed. Out of 110 females and 110 males tested (most in preliminary backcrosses) 60 females and 54 males had the *Dlb-1*^{a} phenotype.

Table 1. *Strain distribution pattern of* Dlb-1, Hba *and* Es-3 *in the CXS recombinant inbred strains (C represents the BALB/c allele, S the STS allele)*

Strain	*Hba*		*Dlb-1*		*Es-3*
1	C	.	C	.	C
2	S	x	C	x	S
3	C	x	S	.	S
4	C	x	S	x	C
5	C	x	S	.	S
6	S	.	S	x	C
7	S	.	S	.	S
8	C	.	C	.	C
9	C	x	S	.	S
10	S	.	S	.	S
11	C	x	S	.	S
12	C	x	S	.	S
13	C	.	C	.	C
14	S	.	S	.	S

For *Hba* typing, the RBC were lysed in 1 ml cold distilled water and frozen at −70 °C. The *Hba* phenotype was then determined according to Whitney *et al.* (1979). The Esterase-3 (*Es-3*) phenotype was determined using starch-gel electrophoresis as described by Roderick, Hulton & Ruddle (1970). In the U.K. the *Es-3* and *Mod-1* phenotypes were determined using cellulose acetate electrophoresis (Hoffman, 1975) of fresh samples of erythrocytes.

In The Netherlands genetic mapping was carried out using mice bred and kept at the animal facilities of the Antoni van Leeuwenhoekhuis. Stock included the CXS set of recombinant inbred strains derived from a cross of BALB/c HeA (C), carrying *Dlb-1*^{b} and STS/A (S), carrying *Dlb-1*^{a} in which the two strains differ in their pattern of lectin binding (Hilgers & Arends, in press). Subsequent crosses involved a double backcross of C57BL/10ScSn × (Gr-*Mtv-2*$^-$/A × C57BL/10ScSn), a three-point linkage triple backcross involving the same strains and BALB/cHeA × (STS/A × BALB/cHeA), and a double backcross of (BALB/cHeA × STS/A) × STS/A.

In the U.K. the SWXL set of recombinant inbred strains kindly supplied by Dr B. A. Taylor (The Jackson Laboratory, Bar Harbor, Maine) was used. These RI strains are derived from a cross of C57BL (*Dlb-1*^{b}) and SWR (*Dlb-1*^{a}). Other crosses involved mice maintained at the MRC Experimental Embryology and Teratology Unit, Carshalton. A number of crosses involving biochemical markers on various chromosomes were used, but as no linkage was found, the results are not reported here. Linkage was eventually detected with *Rex*, a dominant coat texture marker on chromosome 11 (Green, 1981), in a triple backcross involving C57BL/6 × (RIII-*ro* × C57BL/6-*Re*), the results of which are reported here.

Table 2. *Results of crosses involving* Es-3, Dlb-1 *and* Hba *(Netherlands)*

	Offspring phenotypes			
Cross	*Es-3*	*Dlb-1*	*Hba*	No. observed
1	aa	ab	—	4
	aa	bb	—	18
	ab	ab	—	23
	ab	bb	—	7
			Total	52
2	aa	ab	ac	5
	aa	ab	aa	2
	aa	bb	ac	9
	aa	bb	aa	9
	ab	ab	ac	13
	ab	ab	aa	13
	ab	bb	ac	2
	ab	bb	aa	5
			Total	58
3	aa	ab	ba	8
	aa	ab	bb	3
	aa	bb	ba	7
	aa	bb	bb	9
	ac	ab	ba	12
	ac	ab	bb	11
	ac	bb	ba	0
	ac	bb	bb	8
			Total	58
4	—	ab	ba	26
	—	ab	bb	13
	—	aa	ba	14
	—	aa	bb	21
			Total	74

Cross 1.
C57BL/10ScSn × (GR-*Mtv-2*$^-$/A × C57BL/ 10ScSn).
Cross 2.
C57BL/10ScSn × (Gr-*Mtv-2*$^-$/A × C57BL/10ScSn).
Cross 3. BALB/cHeA × (BALB/CHeA × STS/A).
Cross 4. (BALB/cHeA × STS/A) × STS/A.
Note: BALB/cHeA is *Es-3*^{a}, *Dlb-1*^{b}, *Hba*^{b}; C57BL/10ScSn is *Es-3*^{a}, *Dlb-1*^{b}, *Hba*^{a}; GR-*Mtv-2*$^-$/A is *Es-3*^{b}, *Dlb-1*^{a}, *Hba*^{c}; STS/A is *Es-3*^{c}, *Dlb-1*^{a}, *Hba*^{a}.

3. Results

The strain distribution pattern (SDP) of *Dlb-1*, *Hba* and *Es-3* in BALB/cHeA (C) and STS/A (S) and in the fourteen CXS recombinant inbred strains is shown in Table 1. The SDP of 11/14 of the RI strains was concordant between *Dlb-1* and *Es-3*, suggesting that *Dlb-1* is on chromosome 11. However, there was no evidence of linkage with *Hba*, as only 7/14 strains were concordant.

The SDP of the *Dlb-1* locus in the seven SWXL recombinant inbred strains was in exact concordance with *Mod-1* and *Es-13*, on chromosome 9, as reported by Taylor (1981), (i.e. S, L, S, S, S, S, S in strains SWXL 4, 7, 12, 14, 15, 16 and 17, respectively), though a different SDP was recorded for other chromosome

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Table 3. *Estimates of recombination values (Netherlands)*

Loci	Cross	Recombinants	Total	c	S_c
Es-3--Dlb-1	1	11	52	—	—
	2	14	58	—	—
	3	19	58	—	—
	Pooled[a]	44	168	0.262	0.034
Dlb-1--Hba	2	26	58	—	—
	3	21	58	—	—
	4	27	74	—	—
	Pooled[a]	74	190	0.389	0.035
	4	27	74	0.365	0.056
Es-3--Hba	2	32(36)[b]	58	—	—
	3	34(37)[b]	58	—	—
	Pooled[a]	66(73)[b]	116	0.569 (0.629	0.046 0.045)[b]

[a] There was no evidence that the phase of the crosses differed in the rate of recombination, so data were pooled.
[b] (Corrected for the noticed double-crossovers.)

Table 4. *Results of crosses involving* Es-3, Re *and* Dlb-1 *(U.K.)*

	Offspring phenotypes			
Cross	*Re*	*Es-3*	*Dlb-1*	No. observed
1	++	aa	ab	11
	++	aa	bb	1
	++	ca	ab	37
	++	ca	bb	1
	Re+	aa	ab	0
	Re+	aa	bb	42
	Re+	ca	ab	0
	Re+	ca	bb	11
			Total	103
2	++	aa	ab	1
	++	aa	bb	0
	++	ca	ab	18
	++	ca	bb	1
	Re+	aa	ab	0
	Re+	aa	bb	6
	Re+	ca	ab	0
	Re+	ca	bb	3
			Total	29
3	++	—	ab	15
	++	—	bb	0
	Re+	—	ab	2
	Re+	—	bb	11
			Total	28

Cross 1. (RIII-*ro* × C57BL/6-*Re*) × C57BL/6-+.
Cross 2. C57BL/6-+ × (RIII-*ro* × C57BL/6-*Re*).
Cross 3. Similar to cross 1, but not typed for *Es-3*.
Note: RIII is Re^+, $Es\text{-}3^c$, $Dlb\text{-}1^a$; C57BL/6-*Re* is *Re*, $Es\text{-}3^a$, $Dlb\text{-}1^b$.

9 markers by Taylor. However, a double backcross involving 49 mice segregating for *Mod-1* (chromosome 9) and *Dlb-1* and 39 mice segregating for the dilute coat colour (7 cM proximal to *Mod-1*) and *Dlb-1* gave no evidence of linkage ($P > 0.05$). Unfortunately, no data have been published on chromosome 11 markers in the SWXL RI strains.

The results of three experiments carried out in The Netherlands between *Dlb-1* and *Es-3* and *Hba*, both of which are located on chromosome 11 are listed in Tables 2 and 3. In these experiments statistically significant linkage was detected between *Dlb-1* and *Es-3*, and between *Hba* and *Dlb-1*, but not between *Hba* and *Es-3*. This and the three-point linkage experiment suggest a gene order of *Hba---Dlb-1---Es-3*.

The results of a backcross involving *Dlb-1*, *Rex* and *Es-3* carried out in the U.K. are listed in Tables 4 and 5. In this case strong linkage was obtained between *Re* and *Dlb-1*, with weaker linkage between *Dlb-1* and *Es-3*. The three-point linkage experiment suggested a most probable gene order of *Dlb-1---Re---Es-3*. Among the 3 recombinants between *Dlb-1* and *Re* two also showed recombination between *Dlb-1* and *Es-3* but one did not, presumably as a result of a second recombination between *Re* and *Es-3*.

Table 6 summarizes the pooled results from both laboratories, together with estimates of the linkage parameters. The estimates of the linkage parameters are in good agreement with those reported by Roderick & Davisson (1981). It is concluded that *Dlb-1* is situated on chromosome 11, 3.1 ± 1.4 cM proximal to *Re*.

4. Discussion

The linkage determinations presented here were carried out independently in two laboratories, using different techniques and mice, and it is therefore gratifying that they are in such good agreement. The only anomaly of any note is the suggestion from the SWXL RI strains that the *Dlb-1* locus was on chromosome 9.

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Table 5. *Estimates of recombination values (U.K. data)*

Loci	Cross	Recombinants	Total	c	S_c
Re--Es-3	1	23	104	—	—
	2	4	29	—	—
	Pooled[a]	27	133	0.203	0.035
Re--Dlb-1	1	2	103	—	—
	2	1	29	—	—
	3	2	28	—	—
	Pooled[a]	5	160	0.031	0.014
Es-3--Dlb-1	1	24	104	—	—
	2	5	29	—	—
	Pooled[a]	29	133	0.218	0.036

[a] There was no evidence that the phase of the crosses differed in the rate of recombination, so data were pooled.

Table 6. *Summary of linkage parameter estimates*

Loci	Laboratory[a]	Recombination ±S.E.
Es-3--Dlb-1	1	0.262±0.034
	2	0.218±0.036
Dlb-1-Hba	1	0.389±0.035
Es-3--Hba	1	0.569±0.046
Re--Es-3	2	0.203±0.035
Re--Dlb-1	2	0.031±0.014

[a] 1, The Netherlands; 2, United Kingdom.

The change of getting a SDP exactly fitting a chromosome 9 marker among 7 RI strains when the locus is not in fact on that chromosome is 1/128. Thus it is low, but not unduly so. However, these data do help to emphasize that RI strains may give some indication of the linkage of polymorphic markers, but the data must be checked using conventional linkage tests. Had there been some chromosome 11 markers published for this set of RI strains, then it is possible that they would also have given a high level of concordance. As it is, the data presented here provide additional information on the SWXL strains which can be used in the future.

Although the main aim of this paper is to present data showing that the *Dlb-1* locus is on chromosome 11, 3.1 ± 1.4 centrimorgans proximal to *Rex*, it is worthwhile commenting on the rather unusual feature of this locus, namely the reciprocal behaviour of the two alleles. It is relatively easy to understand a locus in which one allele determines the presence, and the other absence of receptors in a specific tissue. However at the *Dlb-1* locus the *a* allele determines the presence of receptors for the lectin in the vascular endothelium, but absence on the gut epithelium, and the *b* allele is exactly the opposite. The F_1 hybrid has receptors in both tissues, but no mouse has yet been found which does not have receptors in one or the other tissue. The results can be partially explained in terms of tissue-specific enzymes, but just why a gene which 'switches on' the mechanism in one tissue should apparently switch it off in a different tissue, remains unexplained. It is hoped that mapping this locus will eventually make it possible to explore the molecular mechanisms of this unusual type of gene action in more detail.

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References

Green, M. C. (1981). Catalog of mutant genes and polymorphic loci. In *Genetic Variants and Strains of the Laboratory Mouse* (ed. M. C. Green), pp. 9-278. Stuttgart, New York: Gustav Fischer-Verlag.

Hilgers, J. & Arends, J. (In the Press). A series of recombinant inbred strains between the BALB/cHeA and STS/A mouse strains. In *The Novel immunological responses of the BALB/c mouse.* (ed. M. Potter). New York: Springer-Verlag.

Hoffman, H. A. (1975). Qualitative characters in genetic monitoring. In *ICLAS Manual for Genetic Monitoring of Inbred Mice* (ed. T. Nomura, K. Esaki and T. Tomita), pp. 38–110. Tokyo: University of Tokyo Press.

Ponder, B. A. J. & Wilkinson, M. M. (1983). Organ-related differences in binding of *Dolichos biflorus* agglutinin to

vascular endothelium. *Developmental Biology* **96**, 535–541.

Ponder, B. A. J., Festing, M. F. W. & Wilkinson, M. M. (1985). An allelic difference determines reciprocal patterns of expression of binding sites for *Dolichos biflorus* lectin in inbred strains of mice. *Journal of Experimental Embryology and Morphology* **87**, 229–239.

Roderick, T. H., Hulton, J. J. & Ruddle, F. H. (1970). Linkage of *Esterase-3* and *Rex* on linkage group VII of the mouse. *Journal of Heredity* **61**, 278.

Roderick, T. H. & Davisson, M. T. (1981). Linkage map. In *Genetic Variants and Strains of the Laboratory Mouse*, (ed. M. C. Green), pp. 279–313. Stuttgart, New York: Gustav Fischer-Verlag.

Taylor, B. A. (1981). Recombinant inbred strains. In *Genetic Variants and Strains of the Laboratory Mouse*, (ed. M. C. Green), pp. 397–407. Stuttgart, New York: Gustav Fischer-Verlag.

Uiterdijk, H. G. & Ponder, B. A. J. (1985). The binding of *Dolichos biflorus* agglutinin to vascular endothelium and intestinal epithelium of mice, a genetic dimorphism. In *Lectins, biology, biochemistry, clinical biochemistry, vol.* 4. (ed. T. C. Bøg-Hansen and J. Breborowicz), pp. 161–168. Berlin: W. de Gruyter.

Whitney, J. B., Copeland, G. T., Skow, L. C. & Russell, E. S. (1979). Resolution of products of the duplicated hemoglobin alpha-chain loci by isoelectric focussing. *Proceedings of the National Academy of Sciences, U.S.A.* **76**, 867–871.

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