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Brief Communications

Linkage of Murine (T,G)-A--L-Specific Idiotypic Determinants to the Heavy Chain Constant Region Allotypic Markers

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We have previously reported the production in guinea pigs of anti-idiotypic antibodies against (T,G)-A--L-specific antibodies of high responder C3H.SW (*H-2*^{b}, *Igh-1*^{a}, Herzenberg and Herzenberg 1978), mice (Schwartz et al. 1978). The guinea pig antisera possessed anti-idiotypic antibodies which reacted with binding-site associated determinants (Schwartz et al. 1978). Immunization with either the closely related antigen (Phe, G)-A--L or with (T,G)-A--L complexed with methylated bovine serum albumin (MBSA) led to the production of (T,G)-A--L-specific antibodies which possess cross-reactive idiotypes with C3H.SW anti-(T,G)-A--L antibodies (Schwartz et al. 1978, Lifshitz et al. 1978). A limited strain survey suggested a linkage between the expression of idiotypic determinants and the heavy chain constant region *Igh-1*^{a} allotypic locus of the mouse (Schwartz et al. 1978). This suggestion was confirmed when (T,G)-A--L specific antibodies of the two congenic strains C3H.SW (*H-2*^{b}, *Igh-1*^{a}) and CWB (*H-2*^{b}, *Igh-1*^{b}), which differ only in the *Igh-1* allotypic complex, were tested. We have demonstrated that, whereas the binding of ^{125}I-C3H.SW anti-(T,G)-A--L antibodies (idiotypes) to guinea pig anti-idiotypic sera was completely inhibited by the homologous antibodies, no inhibition was obtained by excess of CWB anti-(T,G)-A--L antibodies (Schwartz et al. 1978).

In the present report we have broadened the number of strains screened for idiotypic expression utilizing (Phe, G)-A--L to which most of the mouse strains respond with the production of (T,G)-A--L-specific antibodies. In addition, genetic analysis was performed in the segregating backcross population between F_1 hybrids of C3H.SW (idiotype$^+$) and CWB (idiotype$^-$) and the CWB parental mice for establishing the suggested linkage between the expression of the inherited idiotypic determinants and the Igh-1^a allotype.

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The synthetic polypeptides poly (*L*Tyr, *L*Glu)-poly (*DL*A1a)--poly (*L*Lys) abbreviated as (T,G)-A--L and poly (*L*Phe, *L*Glu)-poly (*DL*A1a)--poly (*L*Lys) abbreviated as (Phe, G)-A--L were used as immunogens in this study. The synthesis and characterization of these antigens were previously reported (Sela et al. 1962, Fuchs and Sela 1964).

Different inbred mouse strains were obtained from the Experimental Animal Unit of our Institute and immunized at the age of 2–3 months. (C3H.SW × CWB)F_1 hybrid mice and (C3H.SW × CWB)F_1 × CWB mice were bred and maintained in our laboratory.

Guinea pig anti-idiotypic sera were prepared by repeated immunizations with C3H.SW anti-(T,G)-A--L antibodies in complete Freund's adjuvant (CFA) as previously described (Schwartz et al. 1978). For the preparation of anti-mouse Ig antibodies, guinea pigs were injected twice with purified C3H/He normal Ig in CFA.

(T,G)-A--L-specific antibodies were obtained by immunization of mice with 10 μg of (T,G)-A--L, its MBSA complex or (Phe, G)-A--L emulsified in CFA. Mice were boosted 3 weeks later and bled 8–15 days after the second injection.

Antiserum of LP anti BALB/c myeloma protein (MOPC 74) with antibody activity against *Igh-1*^{a} was a gift from Dr. Rose Lieberman (Laboratory of Immunology, NIH). Determination of allotypes was performed using the immunodiffusion technique in agar gel acording to Ouchterlony (1953).

The binding of ^{125}I-(T,G)-A--L to anti-(Phe, G)-A--L pooled antisera (collected from 20–25 mice), was performed in solution, using the goat anti-mouse Fab sera for precipitating the complex of antigen-antibody as previously described (Lifshitz et al. 1978). The inhibition of the above binding was performed by addition of 5 μl of undiluted guinea pig anti-idiotypic sera to the mouse antisera for 30 min at 37° C before adding the ^{125}I-(T,G)-A--L. For control, 5 μl of guinea pig anti-normal mouse immunoglobulin were used in the inhibition experiments.

Inhibition of binding of ^{125} I-idiotypes to anti-idiotypic sera was performed according to Spring and co-workers (1971). Five μl of mouse immune sera were incubated with 25 μl of the anti-idiotypic sera (in a dilution which yielded 15 percent binding) before the addition of the ^{125}I-C3H.SW anti-(T,G)-A--L antibodies. Precipitates were obtained with goat anti-guinea pig IgG serum. All the inhibition experiments were done in the presence of 25 μl normal C3H.SW sera. The anti-(T,G)-A--L activity of the individual sera used as inhibitors was tested in parallel with ^{125}I-(T,G)-A--L.

We have reported recently (Lifshtiz et al. 1978) that immunization of high (H-2^b) as well as low (H-2^k/H-2^a) responder mice to (T,G)-A--L, with (Phe, G)-A--L led to the production of (T,G)-A--L-reactive antibodies as well as (Phe, G)-A--L unique ones. It has also been shown that in the anti-(Phe, G)-A--L sera of C3H.SW as well as C3H/DiSn only the (T,G)-A--L–specific antibodies but not the (Phe, G)-A--L unique molecules, share the idiotypic determinants with C3H.SW anti-(T,G)-A--L antibodies. This information enabled a broad survey for idiotypic expression in different mouse strains. Results summarized in Table 1 show that the binding of ^{125}I-(T,G)-A--L to anti-(Phe, G)-A--L antisera of different strains congenic for their *H-2* locus (C3H.SW and C3H/DiSn and of BALB.B10 and BALB.C3H) which possess the *a* allelic form of the *Igh-1* locus, was inhibited by the anti-idiotypic sera. On the other hand, the binding of anti-(Phe, G)-A--L sera to ^{125}I-(T,G)-A--L of

Table 1. Association of (T,G)-A--L-specific idiotypes on anti-(Phe, G)-A--L antibodies of different strains with the Ig-1[a] allotypes and not with *H-2* haplotypes

Mouse strain	*H-2* haplotype	Igh-1 allotype	Binding of ^{125}I-(T,G)-A--L (%) in the presence of		Inhibition* (%)
			GPaMIg†	GPaId‡	
C3H.SW	*b*	a	37.4	8.2	78
C3H/DiSn	*k*	a	31.5	9.2	71
CWB	*b*	b	21.6	21.3	1.4
CKB	*k*	b	30.0	33.1	−10.3
BALB.B10	*b*	a	25.4	17.0	33.0
BALB.C3H	*k*	a	16.1	11.7	27.5
C57BL/6J	*b*	b	14.4	13.8	4.2
A/J	*a*	e	32.5	32.0	1.2
DBA/1	*q*	c	36.4	33.7	7.5
C58/J	*k*	a	13.6	15.5	−11.4

* Inhibition of binding of ^{125}I-(T,G)-A--L to anti-(Phe,G)-A--L antisera by guinea pig anti-idiotypic serum. Percent inhibition $= \left(1 - \frac{\text{binding (GPaId)}}{\text{binding (GPaMIg)}}\right) \times 100$.

† Guinea pig anti-mouse immunoglobulin serum.

‡ Guinea pig anti-idiotypic serum.

mice possessing the *b* allelic (CWB, CKB, C57BL/6J), *e* allelic (A/J) or the *c* allelic genes (DBA/1), was not affected by the addition of the anti-idiotypic sera prior to the addition of the ^{125}I-(T,G)-A--L. These results indicate that the (T,G)-A--L site-associated idiotypic expression is linked to the heavy chain allotypic marker, Igh-1[a], and is not controlled by genes in the *H-2* complex. It is demonstrated in Table 1 that anti-(Phe, G)-A--L antisera induced in mouse strains of the C3H genetic background were much more sensitive to the inhibition by anti-idiotypic sera than those of antisera induced in mice of the BALB background. These differences might be due either to the existence of additional idiotypic determinants in the C3H anti-(Phe, G)-A--L antisera which are lacking in antibodies of the BALB mice and/or to differences in the frequency of C3H.SW cross-reactive idiotypes in the (T,G)-A--L specific antibodies of mice of the two backgrounds. It should be noted that the levels of inhibition shown in Table 1 were not affected by the addition of higher doses of the anti-idiotypic serum.

It is also demonstrated in Table 1 that the binding of C58/J anti-(Phe, G)-A--L sera to ^{125}I-(T,G)-A--L was not inhibited by the anti-idiotypic sera. In addition, data demonstrated in Figure 1B show that C58/J antisera elicited by immunization with (T,G)-A--L+MBSA did not compete with the guinea pig anti-idiotypic antibodies on the binding to ^{125}I-idiotypes as compared to the negative controls of 4-hydroxy-5-iodo-3-nitrophenyl-acetyl-ovalbumin (NIP-OVA) and sheep red blood cells (SRBC) specific antisera of C3H.SW (Fig. 1A), which lack the relevant idiotypes (Schwartz et al. 1978). Since C58/J mice possess the Igh-C allotype of C3H.SW mice (Igh-1[a]) but differ in their V_K-*1* allotypic locus (Gottlieb and Durda 1977), now designated *Igk-Trp* locus (Green 1979), these observations suggest that the phenotypic expression of the site-associated (T,G)-A--L idiotypic determinants is affected by the V_L region.

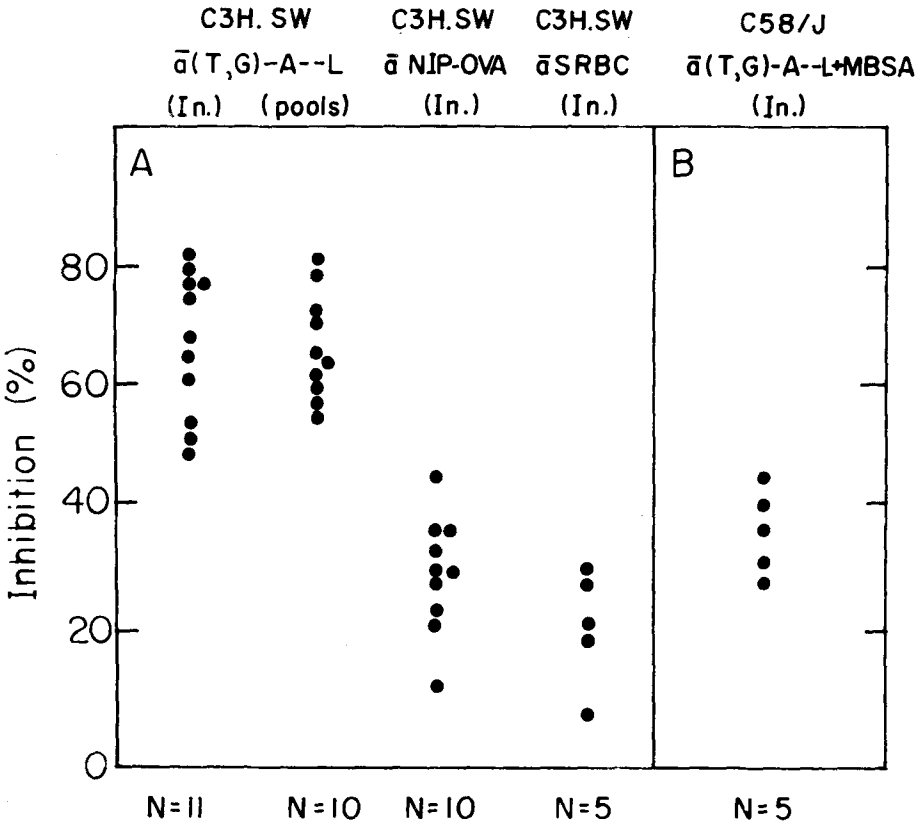

Fig. 1 A and B. Inhibition of the binding of ^{125}I-C3H.SW anti-(T,G)-A--L antibodies to guinea pig anti-idiotypic serum by (**A**), immune sera (pooled and individual, In.) of C3H.SW with different specificities, and by (**B**), sera of C58/J specific to (T,G)-A--L + MBSA. Inhibitions were performed with 5 µl of whole serum in the presence of 25 µl of C3H.SW normal serum.

As seen in Figure 1A, the range of reactivity with anti-idiotypes of pooled sera of C3H.SW mice with anti-(T,G)-A--L activity is comparable to that of anti-(T,G)-A--L sera of individual mice. Thus, the variability of the idiotypic expression in anti-(T,G)-A--L sera of individual mice of the C3H.SW inbred strain is limited.

The linkage between idiotypes and the Igh-1^a allotypic marker was genetically analyzed. (C3H.SW × CWB)F$_1$ hybrid mice were bred with the CWB parental strain. Four groups of individual mice were immunized with (T,G)-A--L. The antisera elicited following immunization were tested for their anti-(T,G-A--L antibody titers and then were independently screened for their allotypes (Igh-1$^{b/b}$ or Igh-1$^{a/b}$) and for their capacity to inhibit the ^{125}I-idiotypes – anti-idiotypes binding. Figure 2 demonstrates that the inhibition of the above binding achieved by antisera of heterozygote mice possessing one set of the *a* allotypic marker (Igh-1$^{a/b}$) were significantly higher than those of the *b* homozygote (Igh-1$^{b/b}$) mice. No correlation was found between the inhibition activity and the anti-(T,G)-A--L antibody titers of

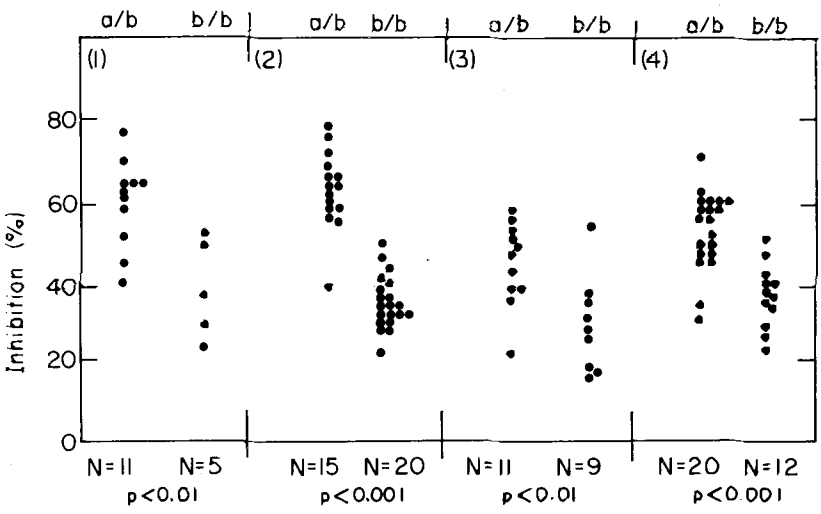

Fig. 2. Inhibition of the binding of ^{125}I-C3H.SW anti-(T,G)-A--L to guinea pig anti-idiotypic sera by serum of (C3H.SW × CWB)F_1 × CWB mice which were immunized with (T,G)-A--L. Experimental groups of mice (1–4) represent offspring of one male and two females, which posses either a/b or b/b heavy chain allotypes. Significant differences *within* the groups between mice possessing the Igh-$1^{b/b}$ and Igh-$1^{a/b}$ allotypes were calculated using the two-tail Student's *t* test.

the antisera tested as demonstrated in Table 2 which represents results with one experimental group. As can be seen in Figure 2, 9 out of 103 antisera tested did not show the expected results (2/5 in group 1, 1/15 in group 2, 1/11 and 1/9 in group 3, 2/20 and 2/12 in group 4). These animals might have been recombinants, since a high rate of recombination has been reported between V_H and C_H or intra V_H determinants (Pisetsky and Sachs 1977). Some of the variations might also be due to the sensitivity of the assay in which whole sera of individual mice were used as inhibitors. However, the results of Figure 2 suggest a linkage between inherited idiotypic determinants specific to (T,G)-A--L and the *Igh-1*^{a} allotypic locus.

These observations agree with those reported by Pincus and co-workers (1978) that Lewis rat antisera raised to anti-(T,G)-A--L antibodies of either C3H.SW or C57BL/10 mice reacted specifically with each of the homologous antibodies. Thus, the demonstration that C3H.SW and C57BL/10 idiotypes specific to (T,G)-A--L are not cross-reactive is consistent with our results that idiotypes of the C3H.SW strains are linked to Igh-1^a allotypes. Indeed, most of the idiotypic systems studied, which are considered as immunoglobulin heavy-chain variable-region loci, *Igh-V* (Green 1979), were found to be linked to heavy chain allotype (Blomberg et al. 1972, Eichmann et al. 1974, Fathman et al. 1977, Mäkela and Karjalainen 1977, and Pawlak et al. 1973). In accordance with the above idiotypic systems (Green 1979), we designate the (T,G)-A--L specific idiotypes of the C3H.SW mouse strain as Igh-Tg. An exceptional system is that of the poly (*L*Glu^{60}, *L*Ala^{30}, *L*Tyr^{10}) (GAT) specific idiotypes which were shown to cross-react with anti-(T,G)-A--L antibodies (Ju and Dorf 1979). These idiotypes were found to cross-react with GAT specific antibodies of all mouse strains tested (Ju et al. 1978).

Table 2. Analysis of anti-(T,G)-A--L titers and idiotypic expression in antiserum of (CWB × C3H.SW)F_1 CWB backcrossed mice

Allotypes a/b (N = 15)		Allotypes b/b (N = 20)	
Antigen binding (%)*	Inhibition of binding of idiotype (%)†	Antigen binding (%)*	Inhibition of binding of idiotypes (%)†
22.3	57	84.8	35
85.4	78	74.6	45
92.6	63	77.1	30
86.0	64	93.0	31
78.6‖	39‖	79.9	36
86.4	77	70.8	31
83.9	70	85.3	36
78.6	62	77.4	47
80.3	60	84.4	28
90.8	66	97.4	31
71.5	59	89.0	42
74.8	66	76.3	26
84.9	74	92.4	39
66.5	59	85.4	33
	63.86 ± 8.7%	82.6	33
	(Mean ± S.D.)‡	82.3	33
		64.2	22
		85.7	26
		67.4	28
		81.1	30
			33.1 ± 6.6%
			(Mean ± S.D.)‡

* Binding (%) of ^{125}I-(T,G)-A--L (5 ng of 2–5 μCi/μg) as determined with 2.5 μl of anti-(T,G)-A--L serum of backcrossed mice.

† Inhibition of ^{125}I-C3H.SW anti-(T,G)-A--L antibodies (idiotypes) binding to guinea pig anti-idiotypic sera by 5 μl of anti-(T,G)-A--L serum of backcrosses (inhibitor).

$$\text{Percent inhibition} = \left(1 - \frac{\text{bound idiotypes (inhibitor)}}{\text{bound idiotypes (no inhibitor)}}\right) \times 100.$$

‡ Percent inhibition, mean ± standard deviation.

‖ Exceptional sample.

The fact that (T,G)-A--L specific sera of C58/J mice bearing the Igh-1^a allotypes of C3H.SW lack the idiotypic determinants of C3H.SW might suggest that the V_L, besides the V_H region, is required for the expression of (T,G)-A--L-site related idiotypic determinants of C3H.SW mice. Laskin and co-workers (1977) showed that idiotypes of anti-p-azophenylarsonate of A/J mice did not only segregate with Igh-C allotypic marker but were also closely linked to the *Igk-1*^{b} allele of the A/J mice and not with *Igk-1*^{a} allele of C58/J mice. It should be noted that the possible role of the V_κ region in the expression of (T,G)-A--L idiotypes is not the only explanation for the lack of reactivity of C58/J (T,G)-A--L specific antibodies with anti-idiotypes. It is also possible that the major idiotypes of C3H.SW (T,G)-A--L specific antibodies will not be expressed in Igh-1^a-possessing mouse strains of all genetic backgrounds as was shown for the anti-nuclease idiotypes of SJL mice. The latter idiotypes which

were shown to be linked to Igh-1^b allotypes of SJL mice were not found on nuclease-specific antibodies of C57BL/10 (Igh-1^b) mice (Fathmann et al. 1977).

In summary, guinea pig antisera raised against anti-(T,G)-A--L antibodies of C3H.SW inbred mouse strain reacted with (T,G)-A--L-site associated idiotypic determinants on (Phe, G)-A--L immune sera of two congenic pairs of mice, high (*H-2*^{b}) and low (*H-2*^{k}) responders to (T,G)-A--L, which differ in their *H-2* haplotype but possess the Igh-1^a allotype. The C3H.SW (T,G)-A--L specific idiotypes were not expressed on antisera of mice bearing the Igh-1^b, Igh-1^c, Igh-1^e allotypes.

C58/J mice (Igh-1^a, Igk-1^a) also lacked the C3H.SW (Igh-1^a, Igk-1^b) idiotypes, suggesting that the V_L region is involved in the expression of the (T,G)-A--L idiotypic determinants.

Genetic analysis of the idiotypic expression in (T,G-A--L-specific antibodies of (C3H.SW $\times$ CWB)F_1 $\times$ CWB backcross mice demonstrated a linkage of the idiotypes to the *Igh-1*^{a} allotypic locus.

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