

derivation in poikilotherms, and such an explanation at present must be purely speculative; it is worth noting, however, that recent investigations on hapten-carrier responses have demonstrated the existence of two cooperating cell populations in the newt, *Triturus viridescens* [19]. The second explanation seems equally plausible: the complete inhibition of antibody responses in thymectomized *Xenopus* may simply indicate that a more effective thymic depletion has been achieved in this animal than in any of the mammalian systems, a factor which might explain, for example, why IgM antibody production in mammals is impaired to a variable extent in different experiments [1, 2, 4, 5, 20].

A further aspect of this problem, and one which has not been examined in the present report, is the extent to which the structural properties of the antigen itself determine the thymic dependence of an antibody response (see [21, 22]). Though the response to SRBC in *Xenopus* mimics "thymus-independent" systems in mammals insofar as antibody produced is exclusively of the IgM type, little is known of the responses of poikilotherms to polyvalent antigens such as lipopolysaccharide or polyvinylpyrrolidone. Attempts to elicit a response to these "classical" thymus-independent antigens in thymectomized *Xenopus* will be of particular interest in this respect.

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Characterization of an immune response gene in mice controlling IgE and IgG antibody responses to ragweed pollen extract and its 2,4-dinitrophenylated derivative*

The strain distribution of IgE and IgG antibody responses to ragweed extract (RE) and its dinitrophenylated derivative (DNP-RE) characterize a new immune response gene, *Ir-RE*. Responder strains show higher primary and secondary levels of both antibody classes than nonresponders after immunization with these antigens in aluminium hydroxide gel, the differences being most marked at low doses, but present over a wide dose range (10 to 500 µg/mouse). High responses are associated with *H-2 a, d, h, k, m, n* and *p* types; low responses with *H-2 b, f, i, j* and *u*. The *Ir-RE* gene has been mapped to the *H-2* region by the use of congenic resistant strains, and to the *H2K -Ir-1* region with *H-2* recombinants. The high response allele is dominant and appears, by its regulation of anti-hapten responses in the DNP-RE hapten-carrier system, to act at the cellular level of thymus-derived lymphocytes. T cells possessing this gene control responses of IgG and IgE-B lymphocytes in a parallel fashion.

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Abbreviations: *Ir*: Immune response *PCA*: Passive cutaneous anaphylaxis, *RE*: Ragweed pollen extract *DNP-RE*: 2,4-Dinitrophenyl conjugate of ragweed extract *DNFB*: 2,4-Dinitrofluorobenzene *T cell*: Thymus-derived lymphocyte *OVA*: Hen ovalbumin *PCA*: Passive cutaneous anaphylaxis

1. Introduction

Genetic control of immunological responsiveness has been demonstrated with a wide variety of antigens. The ability to form specific immune responses to synthetic polypeptides or to limiting doses of certain native protein antigens can be inherited as a single autosomal dominant trait [1, 2]. Genetic analysis of these immune response (*Ir*) genes have generally demonstrated linkage to the major histocompatibility locus of the species [3, 4]. In the present studies, we examine the

immune responses of several inbred mouse strains to ragweed pollen extract (RE) and describe the *Ir-RE* gene which governs the IgE and IgG antibody responses to ragweed extract. This murine model of Ir gene control of responsiveness to ragweed antigens supports previous reports of a similar Ir gene in man [5, 6].

2. Materials and methods

2.1. Animals

Inbred mice of either sex were obtained from Jackson Laboratories, Bar Harbor, Maine or from our own colony. Random bred male Sprague-Dawley rats purchased from Charles River Laboratories, Wilmington, Mass., were used for passive cutaneous anaphylaxis (PCA) assays. All animals used in these studies were between 2 and 6 months of age.

2.2. Antigens

Hen ovalbumin (OVA), 5 x recrystallized was obtained from Pentex Biochemicals, Kankakee, Illinois. Preparations of ragweed pollen extract (RE) (Lot # 36-36-3 x 12) were purchased from Greer Laboratories, Inc., Lenoir, NC. The following 2,4-dinitrophenyl (DNP) conjugates were prepared as previously described [7, 8]: DNP_{4.5} RE and DNP₈ OVA. Subscripts refer to the average number of moles of DNP x 10⁻⁷ per mg of RE protein and moles of DNP per mole of OVA. It is noteworthy that dialysis caused the loss of approximately 75 % of the Kjeldahl nitrogen from the ragweed extract following DNP conjugation.

2.3. Immunization

All immunizations were performed by injecting antigen in 4 mg of aluminum hydroxide gel (alum) intraperitoneally in a total volume of 0.5 ml. Groups of 4-5 mice were primarily immunized with 0.1, 1.0, 10, 100 or 500 µg of RE or 10 µg of DNP-RE on day 0. On either day 15 or day 30, mice were bled and then secondarily challenged with the same antigen and dose. All mice were bled again 7 days after secondary challenge and their sera analyzed for IgE and/or IgG antibodies as described below.

2.4. Antibody titration

2.4.1. IgE antibodies

The levels of reaginic (IgE) anti-RE or anti-DNP antibodies were determined in pooled sera by PCA reactions using Sprague-Dawley rats as previously described [9, 10]. Briefly, 25 µl aliquots of serum from each mouse within a group were pooled and serially diluted (2-fold) in saline. 100 µl of each dilution was injected intradermally into the shaven dorsal skin surface of the test rat. Two to 4 h after intradermal sensitization, RE and DNP-specific PCA reactions were elicited by intravenous injection of 1.0 mg of RE or DNP-OVA, respectively in 1.0 ml of 0.5 % Evans Blue dye. Reactions were read and recorded as the reciprocal of the highest dilution of serum evoking threshold PCA reactivity (5 mm diameter). Sera which failed to elicit detectable PCA reactions were assigned a value of < 10. Two points of importance should be emphasized about this detection system. First, the reaginic antibody activity being measured is completely labile to heating at 56 °C for 2 h, and is detectable 2-4 h after intradermal sensitization of test rats. These are properties of reaginic antibodies of the IgE class and rule out

any contribution of skin-sensitizing antibodies of the IgG₁ antibody class [10]. Second, the DNP-specific nature of these IgE antibodies is verified by the fact that a DNP-conjugate of an unrelated carrier protein (OVA) has been used to test for such antibodies (under conditions where the OVA carrier molecule alone fails to elicit a positive PCA), and moreover, that we have specifically inhibited such PCA reactions with univalent DNP-L-lysine.

2.4.2. IgG antibodies

Serum IgG anti-DNP antibody levels were determined in individual sera by a modified Farr technique using ³H-labeled DNP-ε-amino-N-caproic acid [8]. Using standard curves constructed from specific anti-DNP antibody of corresponding strains of mice, percentage of binding was converted into amount of specific anti-DNP antibody in µg per ml of serum. For statistical analysis, antibody levels of individual animals were logarithmically transformed and geometric means and standard errors were calculated. In those mice in which no significant antibody could be detected in the serum, a value of 0.10 µg/ml was arbitrarily assigned to allow logarithmic transformation of the data. Group comparisons were made employing Student's t-test.

3. Results

3.1. Strain distribution

Table 1 illustrates the PCA antibody responses of 26 different inbred strains to ragweed pollen extract. These strains can be categorized into two groups which differ markedly in their IgE antibody titers to RE. The high responder group, typified by the A/J or B10.D2n strains, displayed variable primary responses and secondary IgE titers of 640 or greater. In contrast, the low or nonresponder strains, eg. C57BL/6 or A.Ca, generally showed no primary response and secondary PCA titers of 40 or less.

Table 1 also demonstrates the association of H-2 type with the level of the anti-RE response. Mice carrying the *H-2 a, d, h, k, m, n* and *p* chromosomal segments fall into the high responder category, while strains bearing the *H-2 b, f, i, j*, and *u* histocompatibility regions are either low or nonresponders to RE. Linkage of the H-2 region with the gene(s) controlling the immune response to ragweed is also demonstrated by the responses of the congenic resistant strains included in Table 1. For example, the congenic resistant strains B10.A, B10.D2n, B10.BR, B10.AKM and B10.F responded well to RE, but the parental C57BL/10 strain and the congenic resistant B10.M and B10.PL were poor responders to ragweed extract. This indicates that the ability to respond to RE was bred into the former congenic resistant strains by the breeding process which put a different H-2 allele on the C57BL/10 genetic background. Conversely, the A and C3H strains were good responders to RE, while their congenic partners A.By, A.Ca and C3H.JK made little or no IgE response to ragweed (Table 1).

3.2. Dose response

Groups of high responder A/J, low-responder C57BL/6 and (C57BL/6 x A/J)F₁ hybrid mice were immunized with 0.1, 1, 10, 100 or 500 µg of ragweed extract. The dose response data shown in Table 2 demonstrate that high responsiveness is a dominant trait and that classification of strains as either high or low responders is possible over a broad range of immu-

Table 1. Strain distribution of IgE responses to ragweed pollen extract^{a)}

Strain	H-2 Type	PCA titers	
		Primary ^{b)}	Secondary
A/J	a	160	2560
B10.A	a	20	1280
C57BL/6	b	<10	40
C57BL/10	b	<10	40
A.By	b	<10	20
BALB/c	d	<10	>2560
DBA/2	d	<10	640
B10.D2n	d	<10	1280
D1.C	d	40	640
A.Ca	f	<10	<10
B10.M	f	<10	<10
B10.A (1R)	h	160	1280
B10.A (2R)	h	160	2560
B10.A (4R)	h	80	1280
B10.A (15R)	h	<10	640
HTI	i	<10	160
B10.A (5R)	i	40	20
B10.A (18R)	i	<10	<10
C3H.JK	j	<10	<10
CBA/HT6	k	1280	2560
C3H/He	k	10	1280
B10.BR	k	20	640
B10.AKM	m	<10	2560
B10.F	n	<10	1280
C3H.NB	p	640	>1280
B10.PL	u	<10	<10

a) All animals were primarily immunized and then challenged on day 30 with 10 µg of RE.

b) Day 30 primary responses.

Table 2. Dose response to ragweed pollen extract

Strain	Immunizing dose (µg)	IgE antibody	
		Primary ^{a)} (PCA titers)	Secondary
A/J	500	640	1280
	100	1280	1280
	10	80	2560
	1	<10	<10
	0.1	<10	<10
C57BL/6	500	<10	160
	100	<10	160
	10	<10	40
	1	<10	<10
	0.1	<10	<10
B6AF ₁	500	80	640
	100	160	1280
	10	40	1280
	1	<10	<10
	0.1	<10	<10

a) Day 15 primary responses.

nizing doses. Priming with 10 to 500 µg of RE elicited primary IgE responses in A/J and F₁ hybrid mice, yet C57BL/6 mice failed to make detectable primary IgE antibodies at any immunizing dose (Table 2). Quantitative differences between the high responder A/J or F₁ mice and the poorly responding C56BL/6 strain were observed during secondary responses in the 10–500 µg dose range. The largest differences in secondary PCA titers were clearly evident at the 10 µg dose; at higher immunizing doses the differences between high and

low responding strains diminished. Immunization with 0.1 or 1 µg of RE failed to elicit IgE antibodies in any of the strains tested.

3.3. Comparison of IgE and IgG responses to DNP-RE

In order to compare the IgG and IgE antibody responses, 7 congenic strains previously characterized in Table 1 for their responsiveness to RE were immunized with 10 µg of the dinitrophenyl derivative of ragweed extract (DNP-RE). IgE antibodies directed against the DNP determinant were measured by PCA assays using DNP-OVA as the challenge antigen; IgG antibodies to DNP were measured by the Farr antigen binding assay. As shown in Table 3, there was an excellent correlation between IgE and IgG anti-DNP antibody levels in both the primary and secondary responses. In addition, the levels of anti-DNP antibodies in various groups correlated with the respective anti-RE antibody responses (Table 3). With the exception of the B10.D2n strain discussed below, the response patterns to DNP-RE followed the same H-2 linked strain distribution shown above for ragweed extract (Table 1).

Interestingly, the secondary IgE and IgG responses of the B10.D2n mice represent an intermediate level of responsiveness compared with either the high responder B10.A, B10.A (2R) and B10.A (4R) lines, or the low responder C57BL/10, B10.M and B10.A (5R) strains. Statistical comparisons of the levels of specific anti-DNP IgG antibodies demonstrated that the B10.D2n response differs significantly from any of the high responder ($P < 0.02$) or the low responder ($P < 0.02$) strains.

Table 3. Comparison of IgE and IgG responses to DNP-ragweed^{a)}

Strain	Primary responses ^{b)}			Secondary responses		
	IgE		IgG ^{c)} (µg/ml)	IgE		IgG ^{c)} (µg/ml)
	RE (PCA titer)	DNP (PCA titer)		RE (PCA titer)	DNP (PCA titer)	
B10.A	160	160	22.1 (1.1)	640	10,240	415 (2.0)
C57BL/10	<10	<10	<0.1	40	<10	0.16 (1.6)
B10.M	<10	<10	<0.1	<10	10	0.16 (1.6)
B10.A (2R)	160	40	2.6 (2.3)	1280	5,120	253 (1.5)
B10.A (4R)	320	80	6.0 (1.2)	>1280	10,240	572 (1.2)
B10.A (5R)	<10	<10	<0.1	<20	160	0.64 (2.3)
B10.D2n	<10	<10	<0.1	320	1,280	19.2 (1.8)

a) Animals were primarily immunized and then challenged on day 15 with 10 µg of RE.

b) Day 15 primary responses.

c) The data are expressed as geometric means of groups of 4–5 mice. Numbers in parentheses denote standard errors.

4. Discussion

This report characterizes a gene(s) that controls the ability of mice to form a specific immune response to ragweed pollen extract or to the DNP-derivative of such extracts. This new immune response gene, termed *IR-RE*, is similar to previously described murine *Ir* genes [4] in that it (a) is autosomal dominant, (b) maps within the major histocompatibility locus and (c) appears to regulate T cell responsiveness. The strain distribution pattern and the responses of the various congenic resistant lines demonstrate that the allele for high level responses to ragweed extract is associated

with the *H-2 a, d, h, k, m, n* and *p* types, while the *H-2 b, f, i, j* and *u* histocompatibility types are associated with relatively poor ragweed responses. This distribution pattern has not been noted for any other Ir gene [4, 11]. More precise mapping of the *Ir-RE* gene can be deduced by studying the response patterns of animals bearing recombinant H-2 regions derived from known crossover events between *H-2a* and *H-2b*. The B10.A (4R) strain, whose high responder *H-2a* chromosomal segment lies to the left of the *Ss-Slp* region, is a high responder to ragweed extract and DNP-RE. In addition, the B10.A (5R) strain whose *H-2a* segment lies to the right of the Ir region of the H-2 complex is a poor responder (Tables 1 and 3). The most interesting recombinant is the B10.A (4R), in which crossing-over occurred within the Ir region [11, 12]. The B10.A (4R) strain localizes the *Ir-RE* gene to the *H2K-Ir-1* (*H2K-Ir-IgA*, *H2K-Ir-1-OA*) region as opposed to the *Ir-IrG* or *Ir-LDH_B* segment of the H-2 complex (see [11, 12 or 13] for additional details).

Genetic control of immune responsiveness in the present study was best observed with limiting doses of immunogen (Table 2), as has been shown with other native protein antigens [2, 14]. Higher doses of ragweed extract may override the genetic control perhaps by allowing successful antigen interactions with cell receptors of lower binding affinity. After mild derivatization with DNP, the ragweed antigenic determinants were still recognized by the high responder strains. Responsiveness to the RE carrier determines that to the DNP hapten as well in low responder strains (Table 3). Since carrier recognition is a function of thymus-derived lymphocytes (reviewed in [15]), the *Ir-RE* gene probably operates on some level in the T cell regulation of immunity. The *Ir-RE* gene affects the IgG and IgE antibody responses in parallel, implying that T cell function at the level of this gene is the same for these two antibody classes, a finding consistent with those of Vaz and Levine [2]. This is an important point in view of the postulated existence of distinct T cells governing the responses of IgG and IgE B lymphocytes, respectively [16].

The studies reported herein demonstrate the presence of a murine histocompatibility-linked immune response gene(s) to ragweed pollen extract, supporting the contentions of

Levine et al. [5] and Marsh et al. [6] that an analogous Ir gene exists in man. Our studies have employed a heterogeneous ragweed pollen extract. The intermediate response of the *H-2d* congenic mice compared to the other responder strains may reflect sensitization to different antigenic determinants or to differences in the Ir genes among responders of various H-2 types, a point that warrants further experimental clarification.

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Complement-independence of tolerance induction

In vivo depletion of plasma complement by the purified C3-cleaving protein of cobra venom (CoF) had no effect on the induction of tolerance in adult mice to either thymus-dependent or thymus-independent antigens. This contrasts with the suppressive effect of CoF on, and the requirement for C3 in, the induction of thymus-dependent antibody production *in vivo* and *in vitro*.

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Abbreviations: CoF: C3-cleaving protein of cobra venom HGG: Human IgG RGG: Rabbit IgG SIII: Type 3 pneumococcal polysaccharide PBS: Phosphate buffered physiological saline, pH 7.2 T cell: Thymus-derived lymphocyte B cell: Bone marrow-derived lymphocyte i.p.: Intraperitoneal i.v.: Intravenous FICA: Freund's incomplete adjuvant SRBC: Sheep red blood cells

1. Introduction

In the past few years there have been several reports that interference with the lytic function of complement may inhibit the induction of tolerance to deaggregated HGG in adult mice [1-4]. Recent *in vivo* and *in vitro* studies have demonstrated that the C3 component of the complement system plays a part in the induction in mice of thymus-dependent, but not thymus-independent antibody production