Immunogenetics 16: 471–483, 1982

Immuno-genetics

Involvement of Mhc Loci in Immune Responses That Are Not *Ir*-Gene-Controlled

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Abstract. Twenty-nine randomly chosen, soluble antigens, many of them highly complex, were used to immunize mice of two strains, C3H and B10.RIII. Lymph-node cells from the immunized mice were restimulated in vitro with the priming antigens and the proliferative response of the cells was determined. Both strains were responders to 28 of 29 antigens. Eight antigens were then used to immunize 11 congenic strains carrying different *H-2* haplotypes, and the T-cell proliferative responses of these strains were determined. Again, all the strains responded to seven of the eight antigens. These experiments were then repeated, but this time antibodies specific for the A ($A_\alpha A_\beta$) or E ($E_\alpha E_\beta$) molecules were added to the culture to block the in vitro responsiveness. In all but one of the responses, inhibition with both A-specific and E-specific antibodies was observed. The response to one antigen (*Blastomyces*) was exceptional in that some strains were nonresponders to this antigen. Furthermore, the response in the responder strains was blocked with A-specific, but not with E-specific, antibodies. The study demonstrates that responses to antigens not controlled by *Ir* genes nevertheless require participation of class II Mhc molecules. In contrast to *Ir* gene-controlled responses involving either the A- or the E-molecule controlling loci (but never both), the responses not *Ir*-controlled involve participation of both A- and E-controlling loci. The lack of *Ir*-gene control is probably the result of complexity of the responses to multiple determinants. There is thus no principal difference between responses controlled and those not controlled by *Ir* genes: both types involve the recognition of the antigen, in the context of Mhc molecules.

Abbreviations used in this paper: A, class II MHC molecule consisting of the A_α and A_β chains; ADH, alcohol dehydrogenase; APC, antigen-presenting cell; cpm; counts per minute; E, class II Mhc molecule consisting of the E_α and A_β chains; *Ir*, immune response; KLH, key-hole limpet hemocyanin; Mhc, major histocompatibility complex; PBS, phosphate buffered salt solution; SD, standard deviation; SI, stimulation index; SN, supernatant of Sendai-virus preparation.

0093-7711/82/0016/0471/$ 2.60

Introduction

When challenged with selected soluble antigens, some guinea pigs and mice mount high, and others low or no immune response in terms of antibody production in vivo (McDevitt and Sela 1965, Benacerraf and McDevitt 1972) or T-cell proliferation in vitro (Shevach et al. 1972, Schwartz and Paul 1976). These differences in immune responsiveness are controlled by immune response or *Ir* loci mapping in the major histocompatibility complex (Mhc; McDevitt and Chinitz 1968). Evidence is accumulating to indicate that the *Ir* genes are in fact identical with class II Mhc genes. The strongest evidence for this identity is the finding that *Ir* gene-controlled proliferation of T cells can be blocked with monoclonal antibodies specific for class II Mhc molecules (Baxevanis et al. 1980, Lerner et al. 1980, Nagy et al. 1981, Nepom et al. 1981). Since the proliferating T cells recognize foreign antigens in the context of class II molecules on antigen-presenting cells (APC) — a situation analogous to that observed with cytotoxic T cells responding to cell-bound antigens (Zinkernagel and Doherty 1974) — their activation can be forstalled by coating the class II molecules of APC with antibodies (Longo and Schwartz 1981, Ishii et al. 1981). The Mhc (class II)-restricted recognition of the antigen by T cells on APC is the first step in a chain of interactions leading, eventually, to antibody production. Thus, the recognition by T cells of a particular combination of antigen and Mhc molecules leads to responsiveness (antibody production), whereas a failure to recognize a particular antigen-Mhc combination results in nonresponsiveness.

If the above interpretation of *Ir* genes is correct, all antigen-triggered, proliferative T-cell responses should be controlled by class II Mhc loci, including those responses in which *Ir* genes are not involved. If all combinations of a given antigen with various Mhc allomorphs (products of allelic genes) were recognized by T cells, all individuals would be responders and genetic analysis leading to the definition of *Ir* genes would not be possible. However, the responses would still involve Mhc molecules in the same way as the responses that *are* controlled by *Ir* genes. To test this postulate, we investigated the context of recognition in proliferative T-cell responses to 29 naturally occurring antigens. The antigens included pathogens (bacteria, viruses, fungi), metazoal parasites or their products, enzymes, hormones, and other proteins, and thus were a representative sample of the antigenic universe. The responses to these antigens were not known to be *Ir* gene-controlled, and yet, as will be demonstrated here, they did involve the recognition of class II molecules.

Materials and Methods

Mice. C3H and B10.RIII mice were purchased, respectively, from the Zentralinstitut für Versuchstiere, Hannover, Federal Republic of Germany, and from Olac, Bicester, Great Britain; all other mice were obtained from the colony in the Max Planck Institute for Biology. Fourteen to 20-week-old female and male mice were used.

Antigens. Alcohol dehydrogenase (yeast), carbonic anhydrase (bovine erythrocytes), catalase (bovine liver), chorionic gonadotropin (human pregnancy urine), creatine phosphokinase (bovine heart), deoxyribnuclease I (DN-100, bovine pancreas), *Escherichia coli* (lyophilized cells of strain W), fibrinogen

(type I, from bovine blood), follicle-stimulating hormone (FSH, porcine), L-glutamic dehydrogenase (bovine liver), β-lactoglobulin A (bovine milk), mucin (neuraminidase substrate, from bovine submaxillary glands), phosvitin (from egg vitelin), protein A (purified from *Staphylococcus aureus*), rennin (chymosin, from calf stomach), ribonuclease B (type III, from bovine pancreas), superoxide dismutase (type I, from bovine blood), thyroglobulin (porcine), trypsinogen (bovine pancreas), and xanthine oxidase (from buttermilk) were purchased from Sigma Chemical Company, St. Louis, Missouri. *Blastomyces* antigen (culture filtrate), *Echinococcus granulosus* antigen (hydatid fluid obtained from fertile sheep cysts), and *Fasciola hepatica* antigen (whole antigen prepared from liver flukes) were obtained from Deutsche Bio-Merieux, Nürtingen, Federal Republic of Germany. Suspension of *Salmonella paratyphi* A-O (group A somatic antigens) was purchased from Wellcome Reagents Ltd., Beckenham, Great Britain. Diphtheria and tetanus toxoid were kindly donated by Statens Seruminstitut, Copenhagen, Denmark. When necessary, the antigens were dialyzed against phosphate buffered saline (PBS). They were dissolved in PBS (2 mg protein/ml), sterilized by γ-irradiation or filtration through 0.45 μm pore-size filters (Milipore), aliquoted, and stored at 20 °C.

Preparation of Sendai virus proteins. Sendai virus was grown in the allantoic cavity of 10-day-old embryonated eggs, purified by sucrose gradient centrifugation, and inactivated with β-propiolactone (Koszinowski et al. 1977). Virus suspended in PBS (10 mg/ml) was solubilized in the detergent octyl-β-D-glucopyranoside (Calbiochem., Giessen, Federal Republic of Germany, final concentration 50 mM) for 30 min at 20 °C. Partial separation of two glycoproteins, fusion protein (F) and hemagglutinin-neuraminidase, from other viral proteins was achieved by centrifugation (100 000 g, 30 min, 4 °C). Supernatant (SN), containing mainly the viral lipids, glycoproteins, and traces of nucleoprotein, and the pellet containing mainly the polymerase, nucleoprotein, and matrix protein, were harvested separately. The detergent was removed from the supernatant and pellet fractions by dialysis against PBS. During dialysis, a high percentage of the glycoproteins spontaneously reconstituted into liposomes (Hosaka and Shinizu 1972). The composition of the three fractions — complete virions, supernatant, and pellet — was determined by the SDS-polyacrylamide gel electrophoresis method of Laemmli (1970) using 5–15% gradient gels.

Monoclonal antibodies. The following antibodies were used: B17-263R1 (Ia.m3), B17-123R2 (Ia.m4), H-116-32R5 (Ia.m6), 13/4 (Ia.m7) (Lemke et al. 1979), 10-3.6.2 (Ia.m17), 11.4.1. (determinant not yet assigned; reacts with the K molecules of *H-2* haplotypes *k*, *p*, *q*, and *r*; Oi et al. 1978), 17-3.3 (determinant not yet assigned; reacts with E molecules of *k* and *r* haplotypes; Ozato et al. 1980), and K25-137 (determinant not yet assigned; reacts with A molecules of *b*, *f*, *j*, *k*, *q*, *r*, and *s* haplotypes; N. Tada, personal communication). To remove any low-molecular-weight, nonspecific, inhibitory substances, the ascites fluids containing the antibodies were fractionated by ultrafiltration using XM-100 A Amicon membrane filters. The antibodies were sterilized by filtration through 0,45 μm pore size filters and stored at −70 °C.

Immunization. The antigens were emulsified in equal volumes of complete Freund's adjuvant (Difco, Hedinger, Stuttgart, Federal Republic of Germany), and 50 μl volumes were injected subcutaneously at the base of the tail (Corradin et al. 1977). The immunzing dose was 50 μg/mouse except for *Blastomyces* antigen (9 μg/mouse) and *Salmonella paratyphi* A-O (2.5×10^8 bacteria/mouse).

T-cell proliferation assay. The standard lymph-node cell proliferation assay (Corradin et al. 1977) was used with slight modification as described previously (Baxevanis et al. 1980). The proliferative response was measured by ^{3}H-thymidin incorporation after three days of culture. All determinations were done in triplicate, and the data were expressed in the Figures as arithmetic mean of cpm ± standard deviation and in the Tables as △ cpm (cpm in cultures with antigen minus cpm in cultures without antigen) and stimulation index (SI, ratio of cpm in cultures with and without antigen).

Inhibition of the response. Monoclonal antibodies were included in the medium and present throughout the culture period. Three to fourfold dilutions of antibodies were prepared with a starting dilution of 1:100. Percent inhibition of the response was calculated using the formula $(A - S/A - M) \times 100$, in which A is cpm in cultures with antigen, S is cpm in cultures with antigen and antibody, and M is cpm in cultures without antigen and antibody.

Results

Proliferative responses of lymph-node cells from C3H mice to 29 naturally occurring antigens

Different doses of antigens were tested for their ability to induce proliferative response of lymph-node cells from immunized C3H mice (Fig. 1), and the optimal dose for each antigen was determined (arrows in Fig. 1). Most antigens elicited good responses in a concentration range from 10 to 500 μg/ml. Some antigens, namely *Blastomyces*, diphteria toxoid, *E. coli*, *Salmonella paratyphi* A-O, and Sendai virus, were toxic for cells at this concentration range, but induced proliferation at lower concentrations (from 0.1 to 5 μg/ml). The response to *Salmonella paratyphi* A-O is not shown in Fig. 1; optimal response to this antigen was obtained when 2.5×10^6 bacteria were added to each well.

All antigens were tested for their mitogenicity, that is, their ability to induce nonspecific proliferation of nonimmune cells. Only Sendai virus, Sendai SN fraction, *Echinococcus granulosus*, *E. coli*, and *Salmonella paratyphi* A-O induced slight nonspecific stimulation (maximum SI = 5); all other antigens failed to stimulate nonimmune cells (SI less than 2; data not shown).

The data in Figure 1 demonstrate that all 29 tested antigens can stimulate in vitro proliferation of lymph-node cells obtained from immunized mice. This proliferation is antigen-dose dependent and specific for the immunizing antigen.

The effect of class II-specific antibodies on the response of lymph-node cells to 27 natural antigens

To determine the involvement of class II Mhc molecules in the response to the tested antigens, we attempted to block the response with monoclonal class II-specific antibodies. To this end, we used immune lymph-node cells from C3H ($H\text{-}2^k$) and B10.RIII ($H\text{-}2^r$) mice and monoclonal antibodies specific for A-molecule determinants Ia.m6 and Ia.m17 and for E-molecule determinants Ia.m7 and 17-3.3; these determinants are expressed by cells of both strains. The blocking effect of antibody K25-137 was also tested in some instances, namely, those in which Ia.m6 and Ia.m17 antibodies blocked the response of B10.RIII cells poorly. Maximal inhibition of the response (obtained at antibody dilutions 1/100 or 1/400) is given in Table 1. With one exception, all the responses were inhibited by both A- and E-specific antibodies. The extent of inhibition varied according to the *H-2* haplotype of the responding mice, the specificity of the antibody used, and the immunizing antigen. Despite these variations, the data shows that both A and E molecules are involved in the proliferative responses to a comparable extent. The only exception was the *Blastomyces* antigen, to which B10.RIII cells did not respond at all; the response of C3H cells was inhibited only with A-specific antibodies.

In determining the involvement of class I molecules, we tested the response of both strains in the presence of a K-specific monoclonal antibody 11-4.1. No significant inhibition was observed, thus indicating that the K molecule does not participate in the responses to the antigens tested (data not shown).

The antibodies were also tested for possible nonspecific inhibitory effects. An example of such a control is shown in Figure 2. Here, a response to the antigen

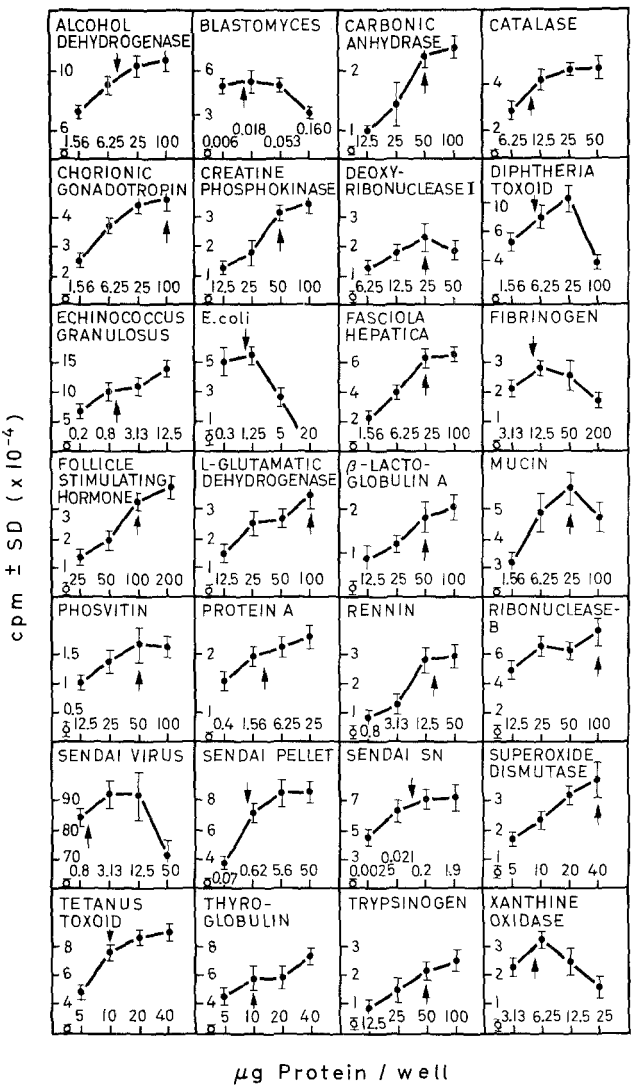

Fig. 1. Proliferative lymph-node cell responses to different doses of 28 antigens (●). The cells were obtained from C3H mice immunized to a given antigen. In control cultures, proliferation was measured in the absence of antigen (o). Vertical lines represent ± SD of triplicate cultures.

alcohol dehydrogenase is elicited in C3H and C57BL/6 ($H\text{-}2^b$) mice. The cells of C3H mice bear determinants Ia.m17 (on the A molecule), Ia.m7 (on the E molecule), and 11-4.1 (on the K molecule). The cells of C57BL/6 mice lack E molecules and hence do not express the Ia.m7 determinant; their A molecules bear the Ia.m3 but not the Ia.m17 determinants. As expected, antibodies specific for the Ia.m17 and Ia.m7 determinants did not inhibit the response of the C57BL/6 mice, and the Ia.m3-specific antibody did not influence the response of the C3H mice. Mixtures of A-specific and E-specific antibodies provide a further control: in C3H mice, their inhibitory effect was stronger than that of the individual A- and E-specific antibodies, but in C57BL/6 mice it did not differ from the pattern of inhibition exerted by the A-specific antibody.

Table 1. Immune response of H-2^k and H-2^r cells to 27 naturally occurring antigens

Antigen	Resp. *H-2* haplo-type	Response		% Inhibition of the response by antibodies specific for			
		△ cpm	SI	A		E	
				Ia.m17	Ia.m6	Ia.m7	17-3.3
Pathogens							
Blastomyces	*k*	40,379	12,33	77	65	0	0
	r	94	1,02	–	–	–	–
Diphtheria	*k*	92,144	27,13	74	65	49	35
toxoid	*r*	76,761	34,95	24	12	0	8
Echinococcus	*k*	84,713	27,29	51	33	48	41
granulosus	*r*	56,991	21,24	23	21	27	30
Escherichia	*k*	108,019	33,23	51	46	50	43
coli	*r*	70,310	20,02	21 (43)*	14	14	18
Fasciola	*k*	55,190	39,87	68	52	66	45
hepatica	*r*	69,365	17,61	52	14	30	28
Protein A	*k*	25,851	17,86	47	74	65	22
	r	10,602	5,16	13 (43)*	0	28	0
Salmonella	*k*	21,565	8,47	75	78	65	44
parathyphi A-O	*r*	32,896	10,70	55	61	29	41
Sendai virus	*k*	90,577	39,59	78	56	56	54
	r	72,221	42,79	29	14	15	18
Tetanus toxoid	*k*	24,110	17,77	46	64	60	50
	r	78,626	48,77	46	28	17	28
Enzymes							
Alcohol	*k*	84,729	30,70	44	21	35	24
dehydrogenase	*r*	25,181	22,36	42	6	26	18
Carbonic	*k*	37,201	20,91	68	51	29	66
anhydrase	*r*	9,265	6,63	0	33	0	55
Catalase	*k*	43,536	13,62	78	54	51	ND†
	r	82,207	13,77	46	31	49	52
Creatine	*k*	36,953	15,58	69	44	86	85
phosphokinase	*r*	57,420	14,87	42	11	41	32
Deoxyribonu-	*k*	29,595	5,10	53	74	50	24
clease I	*r*	15,486	3,83	27 (70)*	26	41	35
L-Glutamyl	*k*	14,615	6,18	52	61	96	72
dehydrogenase	*r*	34,241	17,18	51	17	49	17
Rennin	*k*	58,939	10,58	72	45	52	38
(chymosin)	*r*	61,894	4,92	46	19	41	27
Ribonuclease B	*k*	34,313	18,50	58	64	76	63
	r	28,858	8,82	33	49	75	56
Superoxide	*k*	38,335	30,44	75	73	64	60
dismutase	*r*	33,029	10,84	39	35	33	69
Trypsinogen	*k*	30,364	15,61	71	85	69	56
	r	15,782	6,88	23	60	45	57
Xanthine	*k*	11,259	4,42	52	81	80	60
oxidase	*r*	17,524	7,50	41	45	52	52
Hormones							
Chorionic	*k*	11,732	8,92	101	100	104	96
gonadotropin	*r*	21,938	5,92	67	71	60	84
Follicle-stimula-	*k*	11,936	5,12	66	56	92	71
ting hormone	*r*	34,716	8,65	31	0	31	28
Thyroglobulin	*k*	82,246	30,66	57	64	40	38
	r	12,341	7,70	39	18	48	41

Table 1. (Continued)

Antigen	Resp. *H-2* haplo-type	Response		% Inhibition of the response by antibodies specific for			
		△ cpm	SI	A		E	
				Ia.m17	Ia.m6	Ia.m7	17-3.3
Others							
Fibrinogen	*k*	20,807	19,50	65	71	74	76
	r	3,101	4,86	12	55	63	65
β-Lactoglobu-	*k*	24,133	6,38	53	50	83	51
lin A	*r*	23,826	8,19	17 (27)*	0	49	34
Mucin	*k*	72,168	20,63	65	58	62	60
	r	24,399	7,16	30 (64)*	37	58	55
Phosvitin	*k*	26,363	4,95	99	108	113	110
	r	28,395	9,93	9 (57)*	49	76	85

* The values in parentheses are percent inhibition with monoclonal antibody produced by hybridoma K25-137.

† ND, not done

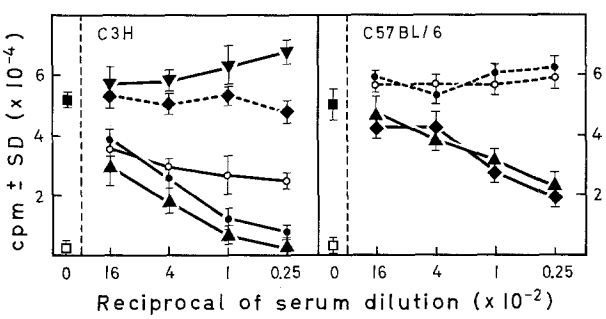

Fig. 2. Inhibition of proliferative lymph node-cell responses. The cells were obtained from C3H or C57BL/6 mice immunized with alcohol dehydrogenase and the cultured cells were restimulated with this same antigen. The antibodies added to cultures were specific for the A (●, anti-Ia.m17, and ◆, anti-Ia.m3), the E (○, anti-Ia.m7), or the K (▼) molecule. The responses indicated by the ▲ signs were those obtained in the presence of mixtures of A- and E-specific antibodies. The solid line indicates that the relevant antigen is expressed, and the broken line indicates that it is not expressed by the responding strain. In control cultures, proliferation was measured without antibodies, in the presence (■) or absence (□) of the antigen. Vertical bars indicate standard deviation.

Mhc involvement in the immune response of cells expressing different H-2 haplotypes

Eleven mouse strains carrying different *H-2* haplotypes on the same (B10) background were tested for their proliferative response to alcohol dehydrogenase, thyroglobulin, diphtheria toxoid, tetanus toxoid (Table 2), Sendai virus, Sendai pellet, and Sendai supernatant (Table 3). All tested mice responded well to all

Table 2. Immune response of 11 congenic mouse strains to alcohol dehydrogensae (ADH), thyroglobulin, diphtheria toxoid, and tetanus toxoid

Mouse strain (*H-2* haplotype)	Antigen	Response		% Inhibition of the response by antibodies specific for	
		△ cpm	SI	A*	E†
C57BL/10Sn (*b*)	ADH	101,901	29,38	49	
	Thyroglobulin	75,204	17,08	61	
	Diphtheria t.	37,102	13,07	52	
	Tetanus t.	55,161	24,01	63	
B10.D2 (*d*)	ADH	69,604	30,09	33	26
	Thyroglobulin	86,635	11,89	56	46
	Diphtheria t.	76,598	23,65	41	38
	Tetanus t.	108,549	44,56	37	33
B10.M (*f*)	ADH	82,445	22,33	39	
	Thyroglobulin	54,575	10,26	72	
	Diphtheria t.	44,962	11,96	41	
	Tetanus t.	65,363	21,46	45	
B10.WB (*j*)	ADH	81,357	18,02	30	47
	Thyroglobulin	58,060	16,86	33	44
	Diphtheria t.	75,648	21,86	51	15
	Tetanus t.	85,699	22,67	36	40
B10.BR (*k*)	ADH	58,199	26,35	45	14
	Thyroglobulin	126,444	12,54	60	23
	Diphtheria t.	61,242	24,00	63	50
	Tetanus t.	53,929	33,41	56	39
B10.P (*p*)	ADH	58,726	18,99	51	46
	Thyroglobulin	79,342	20,04	34	18
	Diphtheria t.	91,524	22,15	36	23
	Tetanus t.	31,345	15,72	59	15
B10.Q (*q*)	ADH	89,187	23,05	42	
	Thyroglobulin	121,524	25,49	43	
	Diphtheria t.	50,709	11,19	72	
	Tetanus t.	53,957	25,85	75	
B10.RIII (*r*)	ADH	39,769	14,62	33	36
	Thyroglobulin	51,542	6,70	29	31
	Diphtheria t.	51,595	17,47	34	8
	Tetanus t.	13,084	6,10	40	20
B10.S (*s*)	ADH	59,145	15,97	95	
	Thyroglobulin	68,353	10,82	71	
	Diphtheria t.	54,646	17,56	90	
	Tetanus t.	77,423	23,68	70	
B10.PL (*u*)	ADH	47,179	18,70	41	44
	Thyroglobulin	64,451	17,06	44	29
	Diphtheria t.	52,463	18,38	53	26
	Tetanus t.	71,225	17,03	47	31
B10.SM (*v*)	ADH	28,132	16,08	47	58
	Thyroglobulin	34,827	13,48	54	58
	Diphtheria t.	46,054	19,94	47	48
	Tetanus t.	50,373	23,00	34	51

* The A molecule was blocked with monoclonal antibodies anti-Ia.m4 (strains C57BL/10Sn, B10.D2, B10.P, B10.S, and B10.SM), anti-Ia.m17 (strains B10.M, B10.BR, and B10.PL), and K25-137 (strains B10.WB, B10.Q, and B10.RIII).

† The E molecule was blocked with monoclonal antibody directed against determinant Ia.m7.

Table 3. Immune response of 11 congenic mouse strains to Sendai virus and its two components

Mouse strain (*H-2* haplotype)	Antigen	Response		% Inhibition of the response by antibodies specific for	
		△ cpm	SI	A*	E†
C57BL/10Sn (*b*)	Virus	104,906	21,64	31	
	Pellet	27,583	14,92	71	
	SN	94,220	30,00	50	
B10.D2 (*d*)	Virus	80,709	37,36	29	30
	Pellet	74,228	30,28	65	59
	SN	91,305	25,45	45	37
B10.M (*f*)	Virus	81,326	17,93	41	
	Pellet	63,964	19,35	58	
	SN	61,521	14,68	49	
B10.WB (*j*)	Virus	68,951	23,15	8	5
	Pellet	53,743	12,40	36	61
	SN	59,748	14,27	36	51
B10.BR (*k*)	Virus	95,591	40,90	46	10
	Pellet	76,279	29,09	64	47
	SN	75,010	39,10	62	36
B10.P (*p*)	Virus	64,104	11,67	46	21
	Pellet	26,402	16,39	73	43
	SN	89,280	19,60	47	54
B10.Q (*q*)	Virus	74,396	21,45	32	
	Pellet	40,344	17,47	64	
	SN	94,500	26,76	57	
B10.RIII (*r*)	Virus	33,455	12,19	5	10
	Pellet	26,470	9,22	39	38
	SN	33,103	12,13	5	23
B10.S (*s*)	Virus	72,718	27,27	34	
	Pellet	69,700	18,16	90	
	SN	76,207	23,09	64	
B10.PL (*u*)	Virus	83,361	31,59	43	29
	Pellet	66,039	18,33	60	47
	SN	92,196	23,29	53	30
B10.SM (*v*)	Virus	48,970	23,68	19	24
	Pellet	52,101	18,67	42	69
	SN	58,696	24,67	40	27

*† Monoclonal antibodies were the same as in Table II.

antigens. The responses were inhibited by both the A- and E-specific antibodies, indicating that both the A and E molecules, when expressed, were equally involved in the responses. A consistent finding was a weaker inhibition of the response to Sendai virus in comparison with the inhibition of the responses to its components, a result possibly related to the larger complexity of the whole virion vis-a-vis its partially purified components (Table 3). Since B10.RIII mice did not respond to the

Table 4. Immune response of 11 congenic mouse strains to *Blastomyses* antigen

Mouse strain (*H-2* haplotype)	Response		% Inhibition of the response by antibodies specific for	
	△ cpm	SI	A*	E*
C57BL/10Sn (*b*)	4,984	1,93	–[†]	·[‡]
B10.D2 (*d*)	144	1,06	–	–
B10.M (*f*)	17,740	6,10	100*	·
B10.WB (*j*)	23,686	15,19	–	–
B10.BR (*k*)	21,115	9,57	65*	0*
B10.P (*p*)	−883	0,83	–	–
B10.Q (*q*)	43	1,02	–	·
B10.RIII (*r*)	−16	0,99	–	–
B10.S (*s*)	1316	1,43	–	·
B10.PL (*u*)	892	1,26	–	–
B10.SM (*v*)	408	1,07	–	–

* To block the A and E molecules monoclonal antibodies specific for the determinants Ia.m17 and Ia.m7, respectively, were used.
† Not done.
‡. The E molecule is not expressed.

Blastomyces antigen and the response of C3H mice was restricted only by the A molecule (Table 1), we tested the response of the eleven congenic mouse strains to this antigen. Table 4 shows that except for strains carrying *H-2* haplotypes *f*, *k*, and *j*, all other strains were nonresponders. The fact that the $H\text{-}2^f$ haplotype, which does not express the E molecule, is a responder confirms that the response is controlled by the A molecule.

Discussion

A major concern in an antibody-blocking experiment is the specificity of the inhibition which must be monitored by a series of controls. The specificity of blocking in the experiments described in this communication is indicated by the following observations. First, irrelevant class II-specific antibodies (i. e., antibodies against determinants not expressed by the particular APCs) had no effect on the response. Second, antibodies specific for class I antigens were used as controls in most of the experiments, but they too had no effect on the response. Third, E-specific antibodies did not inhibit the response in cultures in which the APCs did not express (because of a genetic defect; see Jones et al. 1978) the E molecule. Finally, in related experiments in which *Ir*-controlled responses were tested by the same antibodies as those used in the present study, there was a complete consistency of findings between the genetic mapping and the antibody blocking (Baxevanis et al. 1980, 1981, Ishii et al. 1981). We are therefore confident that our method is reliable, reproducible, and free of artifacts. In the experiments described in this communications — excepting the response to the *Blastomyces* antigen — all strains responded to all the antigens tested; hence none of the responses would normally be

classified as *Ir* gene-controlled. Yet, all the responses were clearly Mhc-controlled, since in all instances the response was blocked by class II-specific antibodies. There is thus no principal difference between responses controlled and not controlled by *Ir* genes; a *seeming* difference arises from some Mhc-antigen combinations failing to stimulate a response with some antigens. These nonresponding strains, however, can then be used for formal genetic analysis of the responsiveness trait.

This conclusion is supported by other studies. Dubriel et al. (1981) described clones of mouse T lymphocytes which responded by proliferation to the antigen keyhole-limpet hemocyanin (KLH) and used the E molecules as restriction elements in this recognition. Clones restricted by the A and E molecule, respectively, were described by Shigeta and Fathman (1981) in studies involving KLH as antigen and by Sprent et al. (1981) for in vivo responses to sheep red blood cells. All these antigens, like those in our study, stimulated responses that are not *Ir* gene-controlled and yet involve the class II Mhc loci. Our data demonstrate the validity of these findings on a large panel of antigens and *H-2* haplotypes.

The only exception in our study was the response to the *Blastomyces* antigen. Some of the strains tested proved to be nonresponders, and if these were used for formal genetic analysis, very likely an Ir gene would be defined controlling the response to this antigen. Since the antigens in this study were selected randomly, it is not surprising that one of them stimulates by chance an *Ir* gene-controlled response.

The question then arises: why do certain antigens stimulate T-cell response in all strains, whereas other antigens stimulate a similar response in only some strains? Several years ago, Benacerraf and McDevitt (1972) pointed out that in *Ir* gene-controlled responses the antigen is either relatively simple, as, for example, one finds with synthetic polypeptides, or is administered in a low dose. In both situations, the antigen confronts the responding T cells with only a few determinants. When more complex antigens or a higher dose of antigen are used, the *Ir* gene control of the response can no longer be demonstrated. Since the antigens used in the present study were deliberately chosen because of their presumed complexity, we assume that the same explanation applies. It is likely that strains not responding to some determinants might respond to other determinants such that the overall phenotype is that of responsiveness. This interpretation is supported by our previous finding that antigens, the response to which is controlled by *Ir* genes, are recognized consistently in the context of one of the two class II molecules [either A or E but never both (Baxevanis et al. 1980, Ishii et al. 1981)]. A similar conclusion can also be extrapolated from the numerous *Ir* gene-mapping studies (reviewed by Krco and David 1981), in which the response could be associated with either the A- or the E-molecule encoding loci but not with both. In contrast to these findings, the present study suggests that in all the strains expressing both the A and the E molecules, the response could be partially blocked by both A- and E-specific antibodies and strongly so by a mixture of these antibodies. Thus, in all tested responses (with the exception of the anti-*Blastomyces* response), at least two antigenic determinants were always involved, one recognized in the context of the A molecule and the other in the context of the E molecule. In fact, the involvement of more than two determinants is very likely. (If only two determinants had been involved, we should have chanced upon strains that were nonresponders to one or the other, or to both determinants.) We suggest, therefore, that the antigens used

here were multideterminant (and some very likely *mixtures* of different antigens) and, as such, stimulated mixtures of A- and E-restricted clones.

The above interpretation of nonresponsiveness could possibly explain the paradox that although the immune system is meant for protection, its repertoire is apparently full of blind spots imposing nonresponsiveness to a great number of antigens. For an individual, the presence of blind spots must be an unfavorable condition and, as such, selected against. Why does it then persist? The answer to this question might be that for most responses the blind spots do not really matter because most natural antigens are complex and the effect of nonresponsiveness to individual antigenic determinants is lost in the general heterogeneity of the response. Only in some instances, when the blind spots concern certain critical determinants present on a pathogen, might the nonresponsiveness lead to a state favoring the development of a disease — perhaps of the kind one finds associated with the Mhc in the humans (Ryder et al. 1979).

The antibody blocking of T-cell responses provides an alternative method to the genetic analysis of the immune response. Its advantage lies in the following: it is fast; it does not involve laborious, time-consuming crosses; and, most importantly, it can be used in situations where no phenotypical difference in responsiveness exists among individuals and inbred strains, i. e., situations precluding the use of formal genetic analysis.

Acknowledgments. We thank Drs. G. J. Hämmerling, D. H. Sachs, and N. Tada for sending us the hybridoma lines, Ms. Catherine Mark for growing the hybridomas and producing the ascites fluids, and Ms. Karina Masur for typing the manuscript. This work was supported in part by Grants Wa 139/10/AI.5 and Na 135/1-1 from the Deutsche Forschungsgemeinschaft.

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Received July 27, 1982