Serological Identification of an Ir-Region Product

Abstract. *Reciprocal immunization of congenic lines differing in the middle portion of the H-2 complex leads to the production of antibodies which react with an antigen or antigens controlled by the Ir region. The antigen designated Ir-1.1 seems to be present only on a subpopulation of lymphocytes from lymph nodes and spleen. It is absent on bone marrow cells.*

The Ir region is a chromosomal segment within the major histocompatability complex of the mouse (H-2). The region has been associated with at least four different functions. It controls the level of antibody response to synthetic polypeptides (*1*) and, apparrently also, to a great variety of other antigens (*2*); it determines the susceptibility and resistance to certain oncogenic viruses (*3*); it plays a major role in stimulation of lymphocytes in mixed lymphocyte culture (MLC) (*4*); and it is significantly involved in graft-versus-host reaction (*5*). A common denominator of these functions is that they all seem to involve, in one way or another, thymus-derived lymphocytes (T cells). It has been speculated that the Ir region codes for the recognition structures on the surface of the T cells (the T cell receptors) (*2*). Since the H-2 complex is not linked to any of the known immunoglobulin loci in the mouse, the implication of this speculation is that the T cell receptor is not an antibody in a classical sense. This conclusion is supported by some findings (*6*) and contradicted by others (*7*).

The discovery of a new class of recognition structures would have an enormous impact on modern immunology. It is therefore of primary importance to identify the products of the Ir region genes. Such identification has been, however, hampered by the fact that none of the functions associated with the Ir region can be used (at least not in a simple way) for assays in biochemical studies. For this reason we attempted to produce antibodies against the hypothetical Ir-region product and to detect the product serologically. Two antiserums to the Ir region are described in this report.

According to the current concept, the H-2 complex consists of four regions: H-2K, Ir, Ss-Slp, and H-2D (*8*). The H-2K and H-2D regions code for histocompatibility antigens responsible for graft rejection and production of humoral antibodies to H-2. All of the known H-2 antigens seem to be determined by these two regions. The Ss-Slp region, located between H-2K and H-2D, codes for serum proteins (*9*). The Ir region is located between the H-2K and the Ss-Slp regions (Fig. 1).

For the production of the antiserums to the Ir region we used strains differing only in the middle portion of the H-2 complex (that is, in Ir and Ss-Slp). The strains were B10.AQR (abbreviated AQR) or H-2^{y-Klj} and B10.T(6R) (abbreviated 6R) or H-2^{y-Sg}. The mode of derivation of the two strains and their H-2 genotypes are shown in Fig. 1. The H-2^{y-Klj} chromosome of AQR was derived by intra–H-2 recombination from chromosomes H-2^a of B10.A and H-2^q of T138 (*10*); the H-2^{y-Sg} chromosome was derived from H-2^a of B10.A and H-2^q of B10.G (*11*). The position of the crossing-over in the two intra–H-2 recombinations is such that the two resulting H-2 chromosomes share their H-2K and H-2D ends, but differ in the central segment of the H-2 complex. The two strains, AQR and 6R, were serologically typed with available antiserums to H-2, which tested for all the major H-2 antigens and were found indistinguishable. Reciprocal immunization of the two strains, however, led to production of antibodies that behaved differently from standard H-2 antibodies.

The mice were originally immunized by repeated injections of combined homogenized spleen, lymph nodes, and thymus (one donor per ten recipients). The thymus was later omitted from the immunizing mixture. The recipients were bled every other day for 3 days, starting 1 week after each injection. The antiserums were tested by the dye-exclusion cytotoxic assay of Gorer and O'Gorman (*12*) in a micro modification according to Amos and co-workers (*13*), and by the indirect fluorescent antibody assay (*14*).

The antibodies in the 6R antiserum to AQR were first detected after the second immunization. The animals then remained positive through at least seven booster injections. In the cytotoxic test, the hyperimmune antiserum gave a positive reaction to a relatively high

Table 1. Cytotoxicity of 6R antiserum to AQR and AQR antiserum to 6R with cells of indicated strains.

Strain	H-2 chromosome	Origin of regions				Cytotoxity (%)*					
						6R antiserum to AQR			AQR antiserum to 6R		
		H-2K	Ir	Ss-Slp	H-2D	1:5	1:10	1:20	1:5	1:10	1:20
B10.AQR	y-Klj	q	k	d	d	50	50	45	0	5	0
B10.T(6R)	y-Sg	q	q	q	d	0	5	0	40	35	40
B10.HTT	tl	s	k	k	d	40	45	35			
B10.S(7R)	th	s	s	s	d	5	5	10			
B10.A	a	k	k	d	d	35	35	35	5	5	0
T138	q	q	q	q	q	5	0	0	35	35	20
B10.G	q	q	q	q	q	5	10	10	30	25	25
A.AL	al	k	k	k	d	40	45	40			
C57BL/10Sn	b	b	b	b	b	5	− 5	5	0	0	5
B10.D2	d	d	d	d	d	10	10	5			
B10.BR	k	k	k	k	k	45	40	35	10	10	10
B10.A(2R)	h-2Sg	k	k	d	b	40	40	40			
B10.A(5R)	i-2Sg	b	b	d	d	− 5	10	0			
B10.AKM	m	k	k	k	q	45	30	35	10	− 5	0
C3H.OL	ol	d	d	k	k	0	0	− 5			
B10.BYR	by	q	k	d	b				5	0	− 5
DA	qs	q	q	q	s	− 10	0	0	25	25	20

* Percentage of dead cells of the indicated strain minus the percentage of dead cells of the recipient strain.

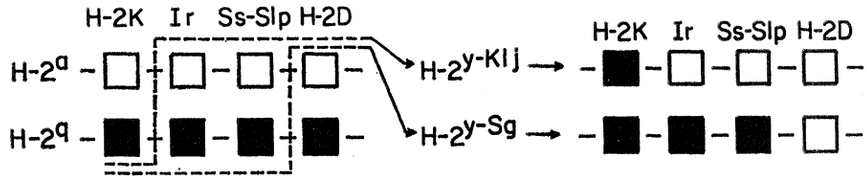

Fig. 1. Genetic origin of chromosomes H-2^{y-Klj} (B10.AQR) and H-2^{y-Sg} [B10.T(6R)]. Dashed line indicates the direction of crossing-over.

dilution (1:1280 to 1:2510). However, even at the lowest dilution (1:2) the antiserum did not kill more than 70 percent of the cell population, which is approximately 45 to 50 percent above the nonspecific background (Fig. 2).

The strains 6R and AQR have the same genetic background, and for this reason the 6R antiserum to AQR should contain only antibodies to H-2. The evidence that this is true comes from testing of the strain T138. Since the B10.A strain was used for the production of both 6R and AQR, the only possible difference between AQR and 6R other than the H-2 difference would have to be introduced into the AQR line by the T138 strain. The 6R antiserum to AQR should, therefore, react with the T138 cells, if it indeed contains other than antibodies to H-2. No such reaction, however, was detected.

As far as can be determined, the H-2 chromosomes of the AQR and 6R strains differ only in the Ir and Ss-Slp regions. The 6R antiserum to AQR should, therefore, contain only antibodies to Ir or to Ss-Slp region (or both). Testing of a selected panel of congenic and H-2 recombinant lines indicates that the antiserum is most likely directed against the product of the Ir region (Table 1). The H-2^a chromosome of the B10.A strain is a postulated recombinant derived from chromosomes H-2^d and H-2^k. The Ir region of H-2^a is derived from H-2^k. Since the H-2^{y-Klj} chromosome inherited its Ir region from H-2^a, the antibody in the 6R antiserum to AQR should be an antiserum to Ir^k. The antiserum should, therefore, react with all strains that carry the Ir^k region. This is indeed the case (Table 1), and the antibody in the 6R antiserum to AQR is not directed against the H-$2K^q$ region (it does not react with B10.G, which carries the H-2^q chromosome) or against the Ss-Slp^q and H-$2D^q$ regions [it does not react with the B10.A(5R) strain, which carries the Ss-Slp and H-2D regions of H-2^a]. Thus, the conclusion seems to be justified that the antiserum detects an antigen controlled by the chromosomal segment between the H-2K and Ss-Slp regions. We call the antigen Ir-1.1.

The tissue distribution of the Ir-1.1 is shown in Table 2. The antigen is present on the lymph node and spleen cells and absent on bone marrow cells. The frequency of Ir-1.1 positive cells in the thymus is very low.

Experiments with lymph node cells from mice thymectomized at birth indicate that the Ir-1.1 is probably present only on T cells. Thymectomy diminished the percentage of cells killed by the 6R antiserum to AQR to the background level. This would explain why the antiserum kills only about 45 to 50 percent of the lymph node and spleen lymphocytes in normal mice.

The reciprocal antiserum, AQR antiserum to 6R, has also been produced (Fig. 2, Table 1). The antiserum should contain antibodies against the Ir and Ss-Slp regions of H-2^q. In agreement with this, the antiserum reacts with B10.G(H-2^q) and DA (H-2^{qs}), which carry the Ir^q and Ss-Slp^q regions; and does not react with B10.AKM(H-2^m) and B10.BYR(H-2^{by}), which carry the H-$2D^q$ and H-$2K^q$ regions, respectively. Thus, the antibody in the AQR antiserum to 6R is apparently directed against an antigen controlled by the chromosomal segment between H-2K and H-2D. In this case, however, it cannot be ruled out that the antibody is antibody to Ss-Slp^q rather than antibody to Ir^q.

A third antiserum which also seems to contain antibodies to Ir has been produced in our laboratory in a combination B10.HTT antiserum to B10.S (7R), or H-2^{tl} antiserum to H-2^{th}. The antiserum has a reaction similar to the 6R antiserum to AQR, thus indicating that it too might be directed against the Ir^k region product.

The Ir-1.1 differs from the H-2 antigens in two important aspects. First, the Ir-1.1 seems to be present only on a subpopulation of lymphocytes (probably only on T cells), whereas the H-2 antigens are present on all cells in a population. Second, the tissue distribution of Ir-1.1 is much more restricted than the tissue distribution of the H-2 antigen. So far, the Ir-1.1 has been detected only on T but not on B lymphocytes, whereas the H-2 antigens are present on both T and B cells. The Ir-1.1, therefore, does not appear to be a typical H-2 antigen.

An important question is whether Ir-1.1 plays any role in the functions ascribed to the Ir region. This can be tested by attempting to block these functions with the antiserums to Ir-1.1. If it can be shown that Ir-1.1 is involved in the T cell recognition process, then, for the first time, a simple assay will be available for biochemical isolation of the recognition structures.

Table 2. Reaction of 6R antiserum to AQR with different AQR cell types in indirect immunofluorescence test.

Source of cells	Fluorescence index (*14*) antiserum dilution:		
	1:5	1:10	1:20
Lymph node	0.58	0.54	0.50
Spleen	0.45	0.35	0.30
Bone marrow	0.02	0.02	0.04
Thymus	0.15	0.10	0.10

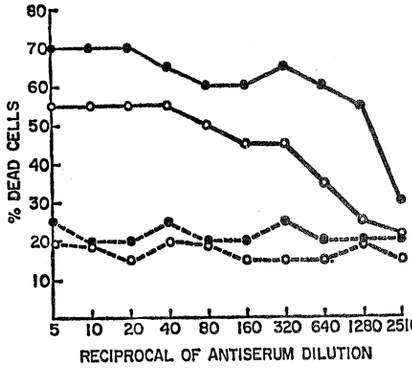

Fig. 2. Cytotoxicity of two Ir region antiserums. The target cells were obtained from lymph nodes. The percentage of dead cells was determined by trypan blue exclusion. ●—●, 6R antiserum to AQR with AQR cells; ● - - ●, 6R antiserum to AQR with 6R cells; ○—○, AQR antiserum to 6R with 6R cells; ○ - - ○, AQR antiserum to 6R with AQR cells.

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Size of the Great White Shark (Carcharodon)

Abstract. *The maximum length of 36.5 feet (11.1 meters) attributed to the white shark (*Carcharodon carcharias*) by Günther and others is a mistake. Examination of the jaws and teeth of the specimen referred to by Günther and comparison with the jaws of white sharks of known length revealed a length of about 17 feet (~5 meters). The largest white shark reliably measured was a 21-foot (6.4-meter) individual from Cuba. Bites on whale carcasses found off southern Australia suggest that white sharks as long as 25 or 26 feet (7½ or 8 meters) exist today. The size of extinct* Carcharodon *has also been grossly exaggerated. Based on a projection of a curve of tooth size of Recent* Carcharodon carcharias, *the largest fossil* Carcharodon *were about 43 feet (~13 meters) long.*

The great white shark, *Carcharodon carcharias* (Linnaeus), is regarded as the most dangerous of all sharks because of its aggressiveness and large size (*1*). In 1870 Günther (*2*) listed jaws of this species (as *C. rondeletii*) from two specimens, *b* and *c*, caught at Port Fairey (?), Australia, and reported to have been 36.5 feet (11.1 m) in length.

Later, Günther (*3*) wrote that the white shark is known to attain 40 feet (12.2 m). Many authorities have given this length or 36.5 feet as the maximum for the species. A few (*4, 5*) have suggested that the 36.5-foot size may represent an example of gigantism.

The second largest white shark believed to be reliably measured was one taken off Cuba that was 21 feet (6.4 m) long (*4*). Why have no white sharks been recorded by actual measurement between 21 and 36.5 feet in length?

I examined the jaws cited by Günther at the British Museum (Natural History). One, marked *c*, consists of only the upper jaw. It measures 1035 mm along the perimeter of the jaw (*6*); the largest tooth is 57 mm in height. The second set of jaws, labeled *b*, is larger (Fig. 1) (*7*). The perimeter of the upper jaw is 1180 mm; the largest upper tooth is 68 mm in height, of which 50 mm is enamel (*8*). Earlier measurements of this tooth have been "nearly 3 inches" (76 mm) (*9*), and "2½ inches" (63.5 mm) (*10*); one author (*11*) stated that *C. carcharias* reaching a length of 35 feet has teeth barely an inch in length.

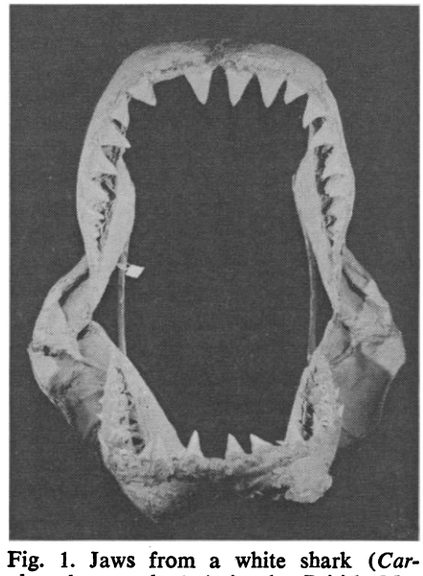

Fig. 1. Jaws from a white shark (*Carcharodon carcharias*) in the British Museum (Natural History) reported as 36.5 feet (11.1 m) in length. The distance between the upper and lower jaws at symphysis (disregarding the teeth) is 593 mm.

Although these jaws are impressive, they do not approach the size one would expect for a shark 36.5 feet long. It is possible that a mistake might have been made in recording the shark's length. P. W. Gilbert independently examined the jaws, and he has suggested that there might have been a printer's error; the length should perhaps have been 16.5 feet (5 m) (*12*). However, Günther still used the 36.5-foot length in the second publication on the subject 10 years after his *Catalogue of Fishes* appeared. Furthermore, he illustrated a tooth from the jaws in natural size (62 mm). This is smaller than the largest tooth, but it corresponds in size to the second largest, which is missing from the upper jaw (see Fig. 1). Nevertheless, Gilbert's postulated 16.5-foot length for the jaws labeled *b* is close to my two estimates, given below.

The vertical height of the enamel of the largest upper tooth and the perimeter of the upper jaw were determined from jaws of white sharks of known length at the Museum National d'Histoire Naturelle, Paris; Scripps Institution of Oceanography, La Jolla; Northeast Fisheries Center, Narragansett; and especially the California Academy of Sciences, San Francisco (jaws collected and prepared by W. I. Follett). In addition, J. T. Veitch of Port Lincoln, Australia, provided measurements from the teeth and jaws of two large sharks. The height of the enamel and measurements of the upper jaw for all these sharks are plotted against total length in Fig. 2, A and B, respectively. From Fig. 2A I estimate a length of 5.6 m (17 feet 9 inches) for a shark with an enamel height of 50 mm, and from Fig. 2B a length of 5.2 m (17 feet) for a shark with an upper jaw perimeter of 1180 mm. I conclude, therefore, that the shark with jaws *b* in the British Museum (Natural History) was about 5.4 m in total length.

Ostle (*13*) reported that the largest white shark taken in the last few years off Western Australia was 19 feet (5.8 m) in length. However, five bites from a larger shark noted on the carcass of a whale which was lost overnight on 26 May 1972 measured 19 inches (483 mm) in height and 24 inches (610 mm) in width. Ostle stated that the bite of a shark of 14.5 to 15 feet long is about 10 by 12 inches, and that