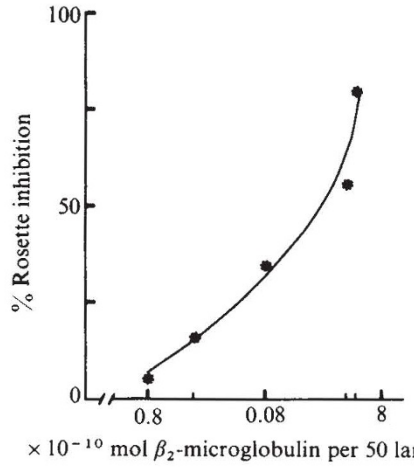

Fig. 2 Inhibitory effect of human β_2-microglobulin on EA-rosette formation. Schistosomula collected *in vitro* after mouse skin penetration were washed three times with large volumes of HLEG. For use in the inhibition assay, 50 larvae were incubated in 1 ml HLEG with different amounts of β_2-microglobulin with frequent mixing at 37 °C for 30 min. Schistosomula were then washed in HLEG and the rosette formation was observed with sensitised sheep RBC (EA) prepared as described in Table 1. Larvae surrounded by less than five red cells on the surface were counted as negative and their percentages were calculated from at least 30 schistosomula.

cercariae did not express these surface receptors, maximum density of binding sites was observed by rosette formation on 3-h-old schistosomula collected after skin penetration. Strikingly, newly transformed schistosomula collected after mechanical tail disruption showed a progressive appearance of binding starting at the anterior end of the parasite, near the glandular apertures, and extending to the whole body within 1 h of *in vitro* incubation. This dynamic process might be related to the excretion of parasite enzymes known to occur during this period[8]. In the later life stages of the parasite, 5-d-old schistosomula and adult worms no longer express the ability to form rosettes.

The binding of IgG to the Fc receptor on eukaryotic cells is not inhibited by β_2-microglobulin despite the homology between this molecule and the third domain of the γ chain. As histocompatility antigens are known to bind to schistosomula[3] we investigated whether β_2-microglobulin interfered with EA rosetting. When schistosomula were first incubated with human β_2-microglobulin (Pharmacia), total inhibition of rosette formation was observed at a concentration of 5 ng ml^{-1} for 30 min at 37 °C (Fig. 2).

The present results indicate that IgG binds to *S. mansoni* schistosomula. The affinity of the receptor for β_2-microglobulin suggests two possibilities: (1) a receptor for IgG which, because of the phylogenic distance between trematodes and mammals presents some unusual features and is mostly for the third domain of the γ chain, or (2), a receptor for β_2-microglobulin binding IgG through the homology with its third domain. In any case, the receptor might be involved in the specific binding of murine major histocompatibility antigens[3] on schistosomula in addition to that of IgG. The precise relationship between these two host antigens is still under investigation.

Table 3 Rosette formation on schistosomula using ox RBC and anti-ox RBC antibody

Ox RBC sensitised with:	Rosetting
Anti-ox RBC IgG (rabbit)	+++
Anti-ox RBC 19S (rabbit)	–
F(ab')$_2$ fragments of rabbit anti-ox RBC IgG*	–

Ox erythrocytes stored in Alsever's solution were washed three times with Hank's balanced salt solution (HBSS) and suspended to 2×10^8 cells ml^{-1} in HBSS. Sensitised ox RBC (EA) were prepared in the usual way with rabbit IgG anti-ox-RBC isolated by column chromatography with DEAE Sephadex A-50, rabbit 19S anti-ox RBC passed through a Sephadex G-200 column and an F(ab')$_2$ fraction prepared by pepsin digestion from rabbit anti-ox RBC IgG.

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Demonstration of MHC-specific haemolytic plaque-forming cells

IT would be convenient to be able to measure the antibody response to alloantigens of the major histocompatibility complex (MHC) at a cellular level. As MHC alloantigens are expressed on erythrocyte membranes in mice one might suppose that a conventional Jerne plaque assay with enumeration of haemolytic plaque-forming cells (allo-PFC) against a lawn of

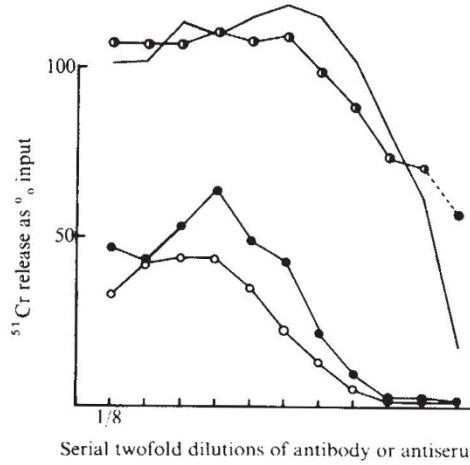

Fig. 1 Synergistic lysis of DA erythrocytes by two monoclonal AO anti-DA alloantibodies, R2/15P (HLGK) and R2/15S (HL) compared with the AO anti DA antiserum S.006. (————). R2/15P (○) and R2/15S (●) were titrated alone, and R2/15P titrated additionally in the constant presence of a 1/32 dilution (final) of R2/15S (◑). Marked synergy between the two monoclonal antibodies is apparent. Their activity together resembles that of the antiserum. Fresh DA erythrocytes were labelled with ^{51}Cr-Na_2CrO_4 as described previously[4]. Guinea pig serum used as a source of complement at 1/8 (final) was absorbed with 2×100% packed rat blood cells before use. The assay mixture was incubated for 1 h at 37°C in V-bottomed microtitration trays.

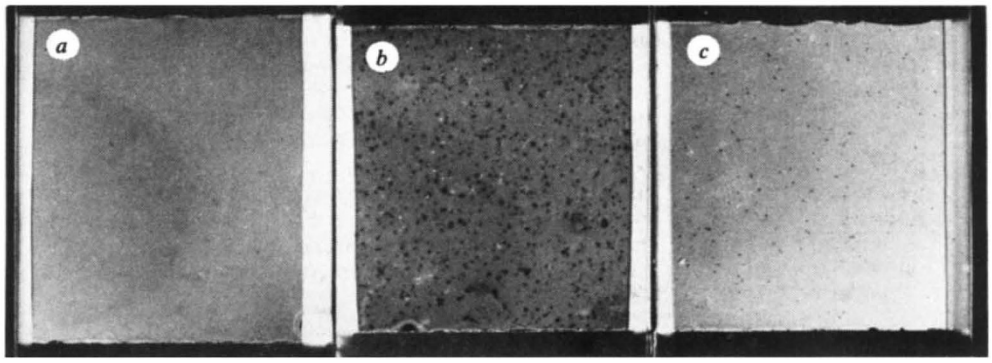

Fig. 2 Plaque formation by spleen cells from an AO rat immunised against DA lymphoid cells (AO 1; see Table 1 legend for immunisation and technical details). 5×10^5 cells per Cunningham chamber: *a*, No additional reagents added to the assay; *b*, R2/10P (HL) monoclonal anti-DA (H-1A^a) antibody added at 1/30 final *c*, R3/47 (HL) monoclonal anti-DA antibody added at 1/10 final. (×2.)

suitable allogeneic erythrocytes would be straightforward. In fact such assays have proved difficult, with very few plaques being detected[1,2]. We have found the same problem in detecting allo-PFC in the spleens of alloimmunised rats. Although alloantisera raised in appropriate strain combinations have a high titre in a direct complement dependent haemolytic assay (Fig. 1) emphasising the potential value of this convenient assay for alloantibody in the rat[3,4], very few allo-PFC were found in spleen cell suspensions from hyperimmunised donors. We show here that the discrepancy may be due to the homogeneity of the antibody product of a single PFC compared with the heterogeneity of serum antibody.

During a study of the haemolytic properties of monoclonal anti-rat MHC (H-1) alloantibodies generated by somatic cell hybridisation[4,5] we observed that of six monoclonal anti-H-1A^a IgG antibodies studied none was able to lyse more than a proportion (<50%) of DA (H-1A^a) erythrocytes at saturation, whereas mixtures of appropriately chosen pairs of monoclonal antibodies would regularly lyse 100% of erythrocytes (Fig. 1). The principal factor limiting cell lysis by a single monoclonal alloantibody was determinant density on the target cell[5]. Recent data demonstrate that erythrocyte populations are heterogeneous in H-1A^a determinant density and that cells with high determinant density are lysed preferentially by a single monoclonal alloantibody (unpublished results). The determinant density dependence of lysis is radically reduced when appropriate mixtures of monoclonal antibodies are used. The definition of 'appropriate' in this context is that the two monoclonal antibodies used should bind noncompetitively to different determinants on the same target molecule. In these conditions, for reasons which are not yet clear, the two antibodies synergise strongly in lytic activity[5].

The failure to demonstrate allo-PFC in the spleens of hyperimmune donors may therefore have been due to the fact that only a small proportion of erythrocytes was lysed by the monoclonal antibody product of each potential plaque-forming cell resulting in 'plaques' that were too faint to be seen. It therefore seemed likely that if one component of a synergistic pair of monoclonal antibodies was added to the plaque assay system in excess, clear plaque formation would occur around a cell secreting a complement fixing antibody that was not competitive with the exogenous antibody. Spleen cells taken from AO (H-1^w) rats 6 and 7 days after an intravenous boosting immunisation with DA (H-1^a) lymphoid cells showed the characteristic low but measurable allo-PFC activity in conventional Cunningham[6] plaque chambers. When exogenous monoclonal anti-H-1A^a antibodies were added to the chambers an enormous increase in plaque count was seen (Table 1, Fig. 2). The size of the increment was a function of the monoclonal antibody added: three monoclonal antibodies probably directed against the same determinant[5] (R2/10P, R2/15P and R3/13) were highly efficient in developing plaques. Monoclonal antibodies directed against another part of the molecule (R2/10S, R2/15S and R3/47) were less efficient. This order has been maintained (with the single exception of AO4) in all animals assayed (Table 1 and unpublished results). It was also noticeable that plaques forming in the presence of R2/10P, R2/15P and R3/13 were larger than those forming with the other antibodies (Fig. 2). It is therefore possible that the differential efficiency is a reflection of differential visibility of plaques. Alternatively, each group of antibodies might allow a distinct subset of the plaque-forming cell response to be seen (that is those producing antibody not competitive with the added monoclonal antibody) and that the subsets are regularly of different sizes.

Table 1 Demonstration of allo-plaque-forming cells using synergistic lysis with monoclonal alloantibodies

	Plaque-forming cells per 10^6 spleen cells						
	Unimmunised AO rats		Immunised AO rats				
Antibody added*	AO(N1)	AO(N2)	1	2	3	4	5
0	<0.1	<0.1	43	97	54	40	20
R2/10P (HL)	–	–	1,950	5,226	3,677	–	–
R2/15P (HLGK)	–	–	2,100	4,548	–	–	–
R3/13 (HL)	0.6	1.8	2,000	3,742	–	480	540
R2/10S (HLK)	–	–	43	742	–	–	–
R2/15S (HL)	<0.1	0.2	825	2,338	2,290	640	240
R3/47 (HL)	–	–	545	1,323	–	–	–
Anti-rat IgG†	–	–	>500	48	26	400	600

AO ♀ rats at 3–4 months old were immunised against DA spleen and lymph node cells by primary subcutaneous and intraperitoneal injection on day 0, followed by an intravenous boosting injection of DA lymphoid cells at day 142 (rat 1), day 43 (rats 2 and 3), or days 43 and 72 (rats 4 and 5). Spleen cells were taken 6 days (rats 1–3) or 7 days (rats 4 and 5) after the i.v. boost, suspended in Dulbecco's phosphate-buffered saline containing 2% fetal calf serum (DAB/FCS), and diluted to an appropriate concentration for assay. Fresh DA erythrocytes were washed in phosphate-buffered saline and diluted to a 25% suspension in DAB/FCS for use. Guinea pig serum absorbed 1:4 with DA blood cells was used as a source of C′ at a final concentration of 1/10. Plaque assays were incubated in Cunningham chambers for 60 min at 37°C.

* Monoclonal antibody supernatants were used at final concentrations between 1/10 and 1/30 in the plaque-forming cell assay with the exception of R2/10S on AO 2. In this case the supernatant was concentrated 10-fold by $(NH_4)_2SO_4$ precipitation between 50% and 60% saturation and used finally at 1/10, equivalent to undiluted supernatant.

† Anti-rat IgG was a rabbit anti-rat IgG2 antiserum (JH 1/7) used at a final (optimal) dilution of 1/30. Other anti-Ig reagents were also used in some experiments. Plaques detected with anti-Ig reagents were always small and hard to score.

Synergistic lysis by monoclonal antibodies is usually a more efficient method of demonstrating allo-plaque formation than is the addition of heterologous antiglobulin antisera (Table 1). Plaques developed by anti-IgG reagents were usually small and hard to score. This low efficiency is presumably partly responsible for the variations recorded in anti-Ig plaque scores in Table 1. We have no direct evidence that the plaques seen in the synergy experiments are produced by IgG antibody. However, since the synergistic lysis phenomenon is only known by us from IgG monoclonal antibodies, and since the assays were performed relatively late (6 or 7 days) in a secondary or tertiary response it seems probable that the exogenous monoclonal antibodies do in fact reveal IgG plaque-forming cells. It may be that the small numbers of plaques seen without the addition of a synergistic monoclonal antibody represent IgM secreting cells. We do not have an IgM anti-H-1A^a monoclonal antibody but such an antibody would be expected to be less dependent on determinant density for its lytic activity. It may be that the H-1A^a specificities are particularly suitable targets for demonstrating the synergistic development of PFC by monoclonal alloantibody, as there is evidence that these specificities are unusually abundant on erythrocytes of the DA strain[7]. However, haemolytic antisera may be raised against other haplotypes[3].

The phenomenon of synergistic lysis explains why only very few allo-PFC could be detected in animals producing high titre haemolytic antisera: a lytic alloantiserum is a synergistic antibody mixture.

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The *beige* mutation in the mouse selectively impairs natural killer cell function

GERM LINE mutations affecting defined cell populations are often valuable tools in elucidating the function of these cells in complex biological systems such as tumour rejection. We report here that a mutant gene in the mouse called *beige* (bg^J), leads to a complete and selective impairment of naturally occurring killer lymphocytes, whereas all other forms of cell-mediated lysis are apparently normal. The defective gene product may lie within the lytic pathway subsequent to tumour cell contact. Because many cell types, including natural killer (NK) cells, T cells and macrophages, may be involved in tumour resistance *in vivo*[1], these mice will provide a critical test of the hypothesis that it is NK cells which provide a first line of defence against neoplasia[2]. It is likely that this mutant will be invaluable for further investigations in tumour immunology just as the nude mouse has been indispensable in evaluating the role of the thymus in the development of the T-lymphoid system and the role of T cells in the rejection of tumours.

The *beige* mutation in C57BI/6 mice occurred spontaneously in an autosomal recessive gene on linkage group XIV (ref. 3) and is thought to provide an accurate model of the Chediak–Higashi syndrome in man[4]. Humoral immunity and delayed-type hypersensitivity are normal and the chief cause of mortality is an increased susceptibility to infection[5] and a lymphocytic infiltration which may be malignant[6].

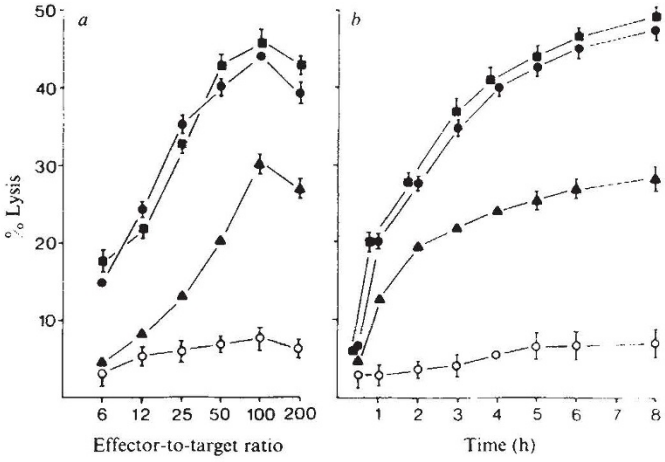

Fig. 1 The absence of NK-mediated cytolysis in *beige* mice. Nylon wool column-passed spleen cells from 4-week-old mice were incubated with ^{51}Cr-labelled YAC tumour cells at various effector-to-target ratios in a 4-h assay[11] (*a*) or for various periods of time at a 100/1 effector/target ratio (*b*). ▲, A/Sn; ○, C57BI/6, bg^J/bg^J; ●, $+/bg^J$ littermates; ■, age-matched +/+ controls. Values represent the mean % specific lysis ±s.e. in quadruplicate wells.

To investigate NK cell function in mutant mice, spleen cells were passed over nylon wool columns, a procedure which enriches for NK cells (Fig. 1). Compared with one of the lowest responder strains known (A/Sn), it can be seen that the homozyous *beige* mutants (bg^J/bg^J) were completely deficient in their ability to lyse YAC cells, a Moloney lymphoma which is the most sensitive NK target yet described. The heterozygous littermate controls ($+/bg^J$) responded as well as the wild type (+/+). The low response of the bg^J/bg^J mice was not altered by prolonged incubation times or high effector-to-target ratios. In additional experiments (unpublished observations) the absence of NK cytolysis was apparent in all the NK-bearing lymphoid organs (spleen, lymph node, bone marrow and peripheral blood) and at all points in the life cycle whether tested against YAC or other NK-sensitive targets (RBL-5, MPC-11, 136-6, P-52, Molt-4). In addition, interferon or interferon-inducing agents (poly I:C, tilorone) failed to augment lysis in *beige* mice (submitted for publication) whereas these agents greatly augmented NK function in low responder strains such as A/Sn[7] and in very young or old mice of the same strain (Gidlund, personal communication). Therefore, the deficient NK response in *beige* mice cannot be accounted for by an altered tissue distribution, age-dependent maturation or a shift in target selectivities. It was then relevant to consider if the defect in cytolysis was selective for NK cells or whether it involved a general failure of all forms of cell-mediated lysis.

As shown in Table 1 (lines 4–6), cytotoxic T lymphocytes generated by alloimmunisation of $+/bg^J$ or bg^J/bg^J mice *in vivo* or by one-way mixed lymphocyte cultures (MLC) *in vitro* were equally capable of lysing P815 cells (a completely NK-insensitive target). In addition, spleen cells from bg^J/bg^J mice alloimmunised *in vivo* responded to secondary challenge in MLC cultures by mounting a strong response on day 3. Peak responses in primary MLC cultures were not obtained until days 5 or 6. Further experiments revealed identical kinetics of cytotoxic