were plugged the first time. Neither owl corrected for the induced localization error. Initially the data from owl 1 suggested some adjustment (Fig. 2*a*), but when the plug was removed after 33 days, the owl immediately localized with normal accuracy (0.0° ± 2.7° and +1.7° ± 2.5°). Similarly, owl 2, whose ear plug was still in place after 77 days, showed no sign of adjusting its error, which remained at L 10.8° ± 2.5° and −9.3° ± 3.7°. The lack of adjustment by owl 2 is particularly revealing because the induced hearing impairment was the same in the first and second experiments (right plug). The owl corrected its error the first time, but not the second. Hence the processes responsible for the adjustment in the first plugging experiment were inoperative in the second, again suggesting that plasticity in the localization mechanism disappears or decreases significantly with age.

During normal growth and development, this capacity for adjustment enables the owl to fine-tune its localization mechanism during the period in life when binaural cues are changing rapidly. After the owl reaches adult size and these cues stabilize, the calibration of the localization mechanism begins to crystallize and finally becomes resistant, if not immune, to further modification by experience.

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Defective T-cell response in beige mutant mice

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A role for T cells in immune surveillance has long been suggested[1], but the lack of high tumour incidence in T cell-deficient nude mice represented a challenge to this hypothesis. The discovery of natural killer (NK) cells, which can lyse tumour cells without any previous sensitization, and high NK levels in nude mice, suggested that these cells may constitute a first line of defence against spontaneously arising tumours *in vivo*[2]. Reports[3–5] of a selective NK deficiency and normal T-cell function in C57BL/6 mice carrying the beige mutation (*bg/bg*) suggested that these mice might be useful for assessing the relative importance of T-cell and NK-cell systems in immune surveillance. However we report here that the deficiency of beige mice is not restricted to NK cells. The generation of cytotoxic T lymphocytes (CTLs) in response to alloimmune challenge *in vivo* or *in vitro* was markedly impaired in beige mutants. Thus our results do not support the suggestion[3,5] that beige mice might be useful as a model of a selective NK deficiency.

To examine the generation of alloimmune CTLs in beige mice, 50 mutant (*bg/bg*) and heterozygous control (*bg/+*) mice ($H\text{-}2^b$) were immunized with a single intraperitoneal (i.p.) injection of P815 ($H\text{-}2^d$) tumour cells. Five mice from each group were killed at different time intervals after immunization and the anti-P815 cytotoxic activities of individual spleen cell preparations were determined at various effector/target ratios in a 4-h ^{51}Cr release assay[6]. Lytic units (LU) of alloimmune CTL activity were determined from the dose–response curves[7]. The P815 subline used in these experiments was not significantly susceptible to lysis by mouse NK cells, target lysis being 0.8–2.8% (effector/target = 100:1) by normal spleen cells from unimmunized mice and 2.5–5.4% by interferon-activated mouse spleen cells[7]. Results (see Fig. 1) indicate that the CTL activity in *bg/+* mice increased rapidly from day 5 and reached a peak value of 33 LU per 10^6 spleen cells on day 11. CTL response was specific to P815 target cells and was not observed against syngeneic EL-4 cells (results not shown). By day 17, the activity had declined considerably but a secondary challenge *in vivo* with 2.5×10^6 P815 cells increased the level of CTLs in *bg/+* mice observed 1 week after the second challenge. At all time points, anti-P815 CTL reactivity was markedly lower in *bg/bg* mice; the peak level of CTLs on day 11 was 11 LU per 10^6 spleen cells. Moreover, a secondary immunization did not increase CTL activity in *bg/bg* spleens. Lytic units in individual spleen cell suspensions indicated considerable variation between animals (Table 1). However, analysis of variance showed that the difference in generation of CTL activity

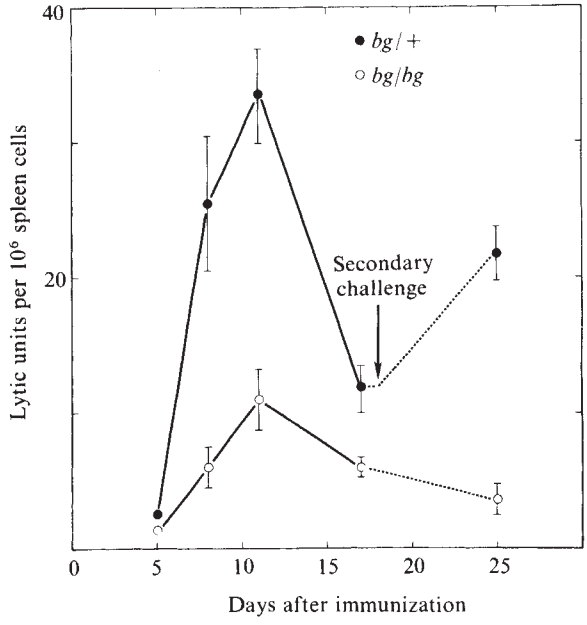

Fig. 1 On day 0, 6-week-old female *bg/bg* or *bg/+* mice were immunized by i.p. injection of 0.2 ml of a P815 tumour cell suspension containing 1×10^7 cells. Tissue-cultured P815 cells used for immunization were washed five times with normal saline before use. On days 5, 8, 11 and 17, five mice each from *bg/bg* and *bg/+* groups were killed and the anti-P815 immune T-cell reactivities of individual spleen cell preparations tested in a 4-h ^{51}Cr release assay at effector/target ratios of 100:1, 50:1, 25:1, 12:1, 12:1 and 6:1 as described previously[6]. Lytic units were calculated from the linear portion of semilog dose–response curves[7]. Each lytic unit represents a target cell lysis of 10% in the test system. Each point in the curve represents the mean ± s.e.m. of lytic units obtained from five spleen cell preparations. On day 18 mice were reinjected with another i.p. dose of P815 tumour cells (2.5×10^6 cells in 0.1 ml saline) and spleen T-cell cytotoxicity was examined on day 25. Spleen cells from unimmunized control mice had no detectable anti-P815 cytotoxic activity.

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Table 1 Analysis of variance for data in Fig. 1

Mice	Lytic units per 10^6 spleen cells*				
	Day 5	Day 8	Day 11	Day 17	Day 25
bg/+	1.86	21.68	32.48	15.44	25.65
	2.46	9.28	38.16	12.48	14.98
	2.32	21.92	36.00	15.12	18.20
	2.68	31.76	41.12	6.64	22.86
	3.56	42.08	20.88	9.68	25.22
bg/bg	1.14	6.28	13.04	2.80	1.69
	1.46	1.36	15.20	4.20	6.46
	0.98	9.96	20.88	6.88	4.28
	1.78	8.56	10.64	5.92	1.43
	1.28	3.86	7.28	5.88	3.87

Source of variation	d.f.	Sum of squares	Mean squares	*F*
Days after immunization	4	2,597.8	649.5	
Beige mutation	1	2,144.2	2,144.2	49.4 ($P<0.001$)
Error (includes animal-to-animal variation)	44	1,911.3	43.4	
Total	49	6,653.3		

* Each lytic unit represents 10% target lysis in the assay system and was computed from the linear parts of individual dose–response curves as described elsewhere[7].

between *bg/bg* and control mice was highly significant ($P<0.001$; Table 1).

Administration of tumour cells may induce two types of lymphocyte-mediated cytotoxic activity in mice. An early augmentation of spleen NK activity peaks on the third day after tumour administration and falls rapidly thereafter to baseline levels by day 6 or 7 (ref. 8). A second peak of specific alloimmune CTL activity occurs later, between days 10 and 11 after immunization[9]. In our experiments, anti-P815 cytotoxic activity of spleen cells was first determined 5 days after immunization and the peak of cytotoxic activity occurred on day 11—a typical CTL response. Treatment of spleen cells with monoclonal anti-Thy-1 antibodies and complement completely abolished their cytotoxicity for P815 target cells, confirming the T-cell nature of the effector cells (results not shown). The monoclonal anti-Thy-1 antibody used has previously been shown to abrogate completely immune T-cell reactivity with no significant effect on the levels of anti-YAC NK activity in spleen cells[10].

Table 2 Generation of cytotoxic T cells in a mixed lymphocyte reaction by *bg/bg* or *+/+* mouse spleen responder cells

Source of spleen cells	Anti-P815 cytotoxicity generated in mixed lymphocyte culture (I.U. per 10^6 spleen cells)
+/+	132.4 ± 13.7
bg/bg	39.2 ± 5.4 ($P<0.001$)

Responder spleen cells were derived from 11-week-old female *bg/bg* or *+/+* mice (C57BL/6). In each experiment, 50×10^6 responder cells were incubated with 10×10^6 X-ray irradiated (1,000 rad) DBA/2 (12-week-old, female) spleen cells for 5 days in conditions described elsewhere[5]. At the end of incubation, cells were washed and tested for cytotoxicity against ^{51}Cr-labelled P815 cells in a 3-h ^{51}Cr release assay at effector/target ratios of 100:1, 50:1, 25:1, 12:1, 6:1 and 3:1. Lytic units were calculated from the linear portion of the dose–response curve as described elsewhere[7]. No significant cytotoxicity was detected in cultures without DBA/2 stimulator cells. Each value is the mean ± s.e.m. of four experiments.

Generation of alloimmune CTLs was also studied in a mixed lymphocyte culture system using a method described elsewhere[5], except that, instead of mitomycin treatment, the DBA/2 stimulator spleen cells were X-ray irradiated (1,000 rad). Results of four experiments (summarized in Table 2) indicate that the levels of cytotoxicity generated by *bg/bg* spleen cells in 5-day MLC were only 30% of control levels. Cytotoxic activity generated in MLC experiments could also be totally abrogated by treatment with anti-Thy-1 + complement (results not shown).

Thus, we could not confirm the presence of a normal T-cell function in *bg/bg* mice; the reason for the discrepancy between our data and those of Roder[3–5] is unclear. The source of *bg/bg* and control mice (Jackson Laboratories) was the same for both studies. A possible explanation is the considerable variation between animals in levels of alloimmune T cells generated in some beige mice, which can approach the lower limits of a normal response (Table 1). This variation was not reported in the previous study[3–5] and could have contributed to the apparent normality of T-cell function observed in *bg/bg* mice; in addition, cells from different mice were pooled and the immune T-cell activity of the pooled spleen cells determined. In certain cases the results of assays using pooled spleen cells may not represent the mean of activities of individual spleen cell preparations[11]. Finally, the data presented by Roder *et al.*[3–5] did indicate a lower than normal level of concanavalin A-induced T cell-dependent cytotoxicity in *bg/bg* spleen cells (Figs 6, 8 of ref. 5).

To compare the magnitude of the CTL induction defect in *bg/bg* mice with the NK cell defect, we attempted to quantify the NK activity in spleen cell preparations derived from *bg/bg* and *bg/+* mice. Anti-YAK NK lytic units per 10^6 spleen cells in *bg/+* spleen cell preparations were 6.26 ± 1.0 (mean ± s.e.m. of eight mice) compared with <0.5 LU in *bg/bg* mouse spleen cells. These results confirmed the previously reported NK defect in *bg/bg* mice[3–5].

Thus we have demonstrated a marked defect in beige mice in the generation of alloimmune T cells both *in vivo* and *in vitro*. Whether the defect in T-cell response in *bg/bg* mice is generalized is unknown. Significantly lower than normal CTL response to lymphocytic choriomeningitis virus infection has been demonstrated in *bg/bg* mice[12], whereas the response to vesicular stomatitis virus was approximately normal[13]. It is therefore possible that a normal or a subnormal CTL response in *bg/bg* mice may depend on the antigen used. Our preliminary findings stress the need for caution when using *bg/bg* mice as a model for a selective NK deficiency with normal T-cell function. Small but significant differences in resistance to certain leukaemias between *bg/bg* and *bg/+* mice[14] could result from a NK-cell defect or T-cell defect or both.

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