Table 2 NK cell activity in normal or cultured spleen and bone marrow cells (BDF_1 mice)

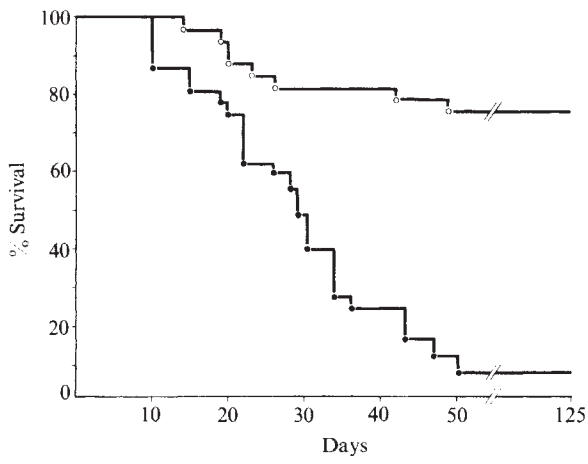
Effector: target cell ratio	Normal spleen Expt 1	Normal spleen Expt 2	Cultured spleen non-adherent cells 24 h Expt 1	Cultured spleen non-adherent cells 24 h Expt 2	Cultured spleen non-adherent + adherent cells 24 h Expt 1	Cultured spleen non-adherent + adherent cells 24 h Expt 2	Cultured spleen non-adherent + adherent cells 7 d	Normal bone marrow	Cultured bone marrow 7 d
100:1	27.0	30.7	2.8	1.9	1.1	2.3	6.8	12.0	3.3
50:1	13.7	16.7	1.9	1.0	1.2	1.1	3.2	6.5	0.8
25:1	9.4	7.7	1.7	1.1	1.1	1.5	6.0	3.4	0.0

Spleen cultures were established by inoculating 10^8 spleen cells into culture flasks containing 10 ml of growth medium[1]. Various times later the non-adherent cells were collected and assayed separately, or as a mixture, with the adherent cells for NK activity. Bone marrow cultures were established in the conventional way[1] and assayed 7 d after inoculation of the second bone marrow population. NK cell activity was measured by the cytotoxicity of spleen or bone marrow cells against ^{51}Cr labelled K562 human CML in blast crisis target cells. 10^4 K562 and varing numbers of effector (marrow or spleen) cells were incubated overnight and the ^{51}Cr release from the cells measured according to published procedures[22]. Similar results were obtained when YAC-1 target cells were used.

in vitro[16,17]. The cell type involved in this type of resistance, therefore, seems to be distinct from NK cell or H-v-G activity.

The possibility that cultured stem cells could be used to reconstitute the haemopoietic system of irradiated allogeneic mice, without the development of H-v-G disease, was indicated by our finding that T lymphopoiesis was not maintained in the cultures (since T lymphocytes are the effector cells involved in the H-v-G response[18]). Figure 1 demonstrates that 90% of BDF_1 mice, injected with normal C57BL/6 bone marrow cells, died within 70 d post reconstitution. On necropsy the mice had grossly enlarged spleens indicative of G-v-H disease[12,19]. At this time only 20% of mice reconstituted with cultured C57BL/6 stem cells had died—with most of the deaths occurring in the first 4 weeks after reconstitution. At 120 d after reconstitution, some mice from this group were sacrificed, and the femoral marrow cells were assayed for CFU-S using irradiated BDF_1 and C57BL/6 mice as recipients. Spleen colonies developed only in the latter strain, showing that the cultured C57BL/6 marrow cells had indeed reconstituted the original BDF_1 recipients. Injection of cultured bone marrow cells, therefore, gives a situation analogous to that seen following reconstitution of irradiated mice with marrow cells which have been selectively depleted of T lymphocytes by immunological techniques[18,20].

Fig. 1 Survival of irradiated BDF_1 mice reconstituted with normal (●) or cultured (○) C57BL/6 bone marrow cells. C57BL/6 bone marrow adherent layers, established for three weeks, were 're-charged' with 10^7 C57BL/6 bone marrow cells. The cultures were fed weekly by demi-depopulation. Two and three weeks later, the cells were collected and 5×10^6–10^7 cells containing approximately 750–1,500 haemopoietic stem cells or CFU-S[21] were injected into irradiated (800 rad X rays) BDF_1 mice. Control mice received 5×10^6–10^7 freshly isolated (not cultured) C57BL/6 bone marrow—containing approximately equal numbers of CFU-S. Each group comprised 40 animals.



The significance of the present observations lies in the possibilities of establishing human cultures using allogeneic bone marrows and the potential of exploiting such cultures for the production of stem cells for marrow transplantation purposes (without the disadvantages of developing G-v-H disease). Furthermore, the absence of mature B, T and NK cells, in the murine system is allowing work on the genesis of these cells from the pluripotent haemopoietic stem cell.

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Hepatic glucocorticoid receptors and the *H*–2 locus

VARIOUS inbred strains of mice exhibit different degrees of susceptibility to both spontaneous and glucocorticoid-induced cleft palate[1,2], and specific genetic and biochemical factors that may be responsible for these differences have been identified. At the genetic level, variations at the *H*–2 locus have been associated with susceptibility to glucocorticoid-induced cleft palate[3,4], while at the biochemical level, evidence suggests that numbers and characteristics of glucocorticoid receptors in murine embryonic facial mesenchyme cells differ in the various

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strains. Specifically, Salomon and Pratt showed that facial mesenchyme cells cultured from fetuses of the A/J strain (which is highly susceptible to glucocorticoid-induced cleft palate) contain twice as many glucocorticoid receptors as do such cells from C57BL/6J, a low susceptibility strain[5]. They also reported that the glucocorticoid receptor of the A/J strain has a lower affinity for dexamethasone ($K_D = 1{\cdot}7 \times 10^{-8}$ M) than does that of the C57BL/6J strain ($K_D = 7.9 \times 10^{-9}$ M) (ref. 5). In an attempt to relate steroid receptor activity to *H*-2 haplotype, Goldman *et al.* used ^{3}H-cortisol and isoelectric focusing to study glucocorticoid receptors in facial mesenchyme cells of these and several other strains[6]. They concluded that "a product of a gene in or near the *H*-2 locus seems to be the glucocorticoid receptor, the level of which must be increased for a congenital malformation to occur". Such conclusions must be questioned, however, because the experimental procedure required micro-electrofocusing of isolated protein–cortisol complex for 40 h. Receptor binding is reversible, and in the absence of free steroid, the half life of the receptor–cortisol complex is only about 1 h at 0 °C (ref. 7). It is unlikely that a peak of radioactivity detected by isoelectric focusing after 40 h represents binding to a specific receptor. Thus an association between *H*-2 haplotype and glucocorticoid receptor activity has not been established definitively. However, the possibility that *H*-2 might determine hormone receptor levels or characteristics is exciting. We have explored the relationship between *H*-2 and glucocorticoid receptors by examining the level of specific glucocorticoid-binding activity in liver cytosols prepared from different mouse strains.

Liver is suitable for such studies because liver cytosol preparations tend to have a relatively stable glucocorticoid-binding activity[8,9], while unbound glucocorticoid receptor molecules undergo rapid, temperature-dependent inactivation in most other cell-free systems. The liver is also well characterised as a target of glucocorticoid action, and *H*-2 is expressed in this organ[10]. Thus an effect of *H*-2 haplotype on the amount or affinity of specific glucocorticoid binding should be observed in this tissue. We examined specific glucocorticoid binding in the two strains studied by Salomon and Pratt, A/J (high susceptibility) and C57BL/6J (low susceptibility), as well as in the two *H*-2 congeneic lines A.BY and B10.A.

Because glucocorticoid-receptor inactivation is greatly accelerated at elevated temperatures[7], we took care to prepare and maintain the liver cytosol at 0 °C. Adult males were killed by decapitation. Livers were removed and chopped on glassine paper over dry ice, weighed and transferred to a chilled homogeniser. Four volumes of ice-cold buffer (10 mM Tris, *p*H 7.35, 0.1 mM EDTA) were added and the tissue was homogenised on ice with nine strokes of a motor-driven Teflon pestle. The homogenate was centrifuged at 35,000*g* for 30 min, the supernatant was removed and assayed for glucocorticoid-binding activity.

Figure 1 shows binding of ^{3}H-TA to the macromolecular component of liver cytosol as a function of free steroid concentration for the A/J and C57 strains. Nonspecific binding (binding of ^{3}H-triamcinolone acetonide (TA) in the presence of an excess of cold dexamethasone) is the same in both strains, whereas total binding is significantly higher in A/J. Specific binding (total less nonspecific, upper inset, Fig. 1) is about twice as high in A/J as it is in C57. Scatchard plots of the data (Fig. 1, lower inset) suggest a single class of high-affinity binding sites. The initial slopes, representing specific binding, are approximately equal, giving an apparent K_D of $1{\cdot}8 \times 10^{-8}$ M for both the A/J and C57 strains. Experimentally determined values for specific binding and apparent dissociation constants can be affected significantly by inactivation of the unbound receptor. Rates of receptor inactivation must be similar in cytosol preparations from the two strains before values for dissociation constants and specific binding can be usefully compared[11]. Figure 2 shows the decline of specific binding activity observed when liver cytosol is incubated in the absence of free steroid for increasing periods. Rates of inactivation of unbound receptors

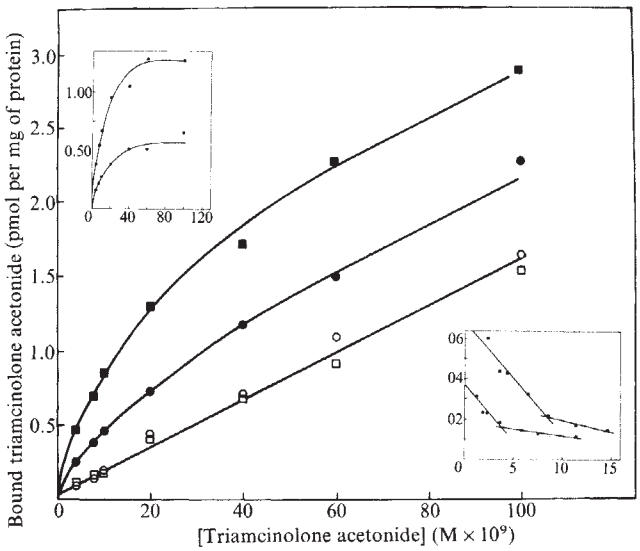


Fig. 1 Binding of ^{3}H-TA presented as a function of free steroid concentration. Samples of 0.2 ml of liver cytosol were incubated in a total volume of 0.5 ml for 22 h at 0 °C with various concentrations of ^{3}H-TA (21.6 Ci mmol^{-1}) in the presence of vehicle or a high concentration (5×10^{-5} M) of competing, nonradioactive dexamethasone. The bound steroid was separated from the free compound by passage through columns (1 cm × 22 cm) of Sephadex G-25 with an elution buffer of 0.01 M Tris (*p*H 7.35), 0.04 M KCl. The columns were run at 4 °C. Fractions (1 ml) were collected and the macromolecular peak was identified by the presence of blue colour resulting from the addition of two drops of a solution of blue dextran to each sample immediately prior to application to the column. Each point represents the binding expressed as pmol bound per mg of protein. Binding in the presence of vehicle: ■, A/J; ●, C57BL/6J; binding in the presence of nonradioactive dexamethasone (nonspecific binding); □, A/J; ○, C57BL/6J. The upper inset shows specific binding (total minus nonspecific) for each strain. Bound TA (pmol per mg of protein) is plotted against unbound TA (M × 10^9). The lower inset presents Scatchard plots of the binding in the presence of vehicle. The ratio of bound to unbound TA is plotted against bound TA (M × 10^{10}).

are the same in both strains. These rates and the observed levels of glucocorticoid binding were not affected by dithiothreitol.

To determine whether or not differences in glucocorticoid-receptor activity observed in the two strains are mediated by the *H*-2 locus, activities of glucocorticoid receptors in the two congeneic lines, A.BY and B10.A, were determined. A.BY has the $H\text{-}2^b$-region of the C57 strain placed on the A genetic background, while B10.A has the C57 background, but the A/J $H\text{-}2^a$-region. The results are shown in Table 1. The mean specific binding capacities in the four strains are significantly different ($P < 0.002$), however the difference is due to genetic background ($P < 0.001$) and not *H*-2 ($P = 0.99$). There is some possibility of an *H*-2 × background interaction effect ($P = 0.10$), which in this case would mean that the effect of genetic background on levels of glucocorticoid receptors is more pronounced in the presence of the $H\text{-}2^b$-haplotype than in the presence of the $H\text{-}2^a$-haplotype.

Thus we have shown that (1) rates of glucocorticoid-receptor inactivation are the same in both A/J and C57BL/6J mice; (2) affinities of the receptors for TA are the same in both strains; (3) A/J has approximately twice as many glucocorticoid receptors as C57BL/6J, and (4) this difference is not determined by variations at the *H*-2 locus.

It is interesting to compare our results with what is known about spontaneous and glucocorticoid-induced incidence of cleft palate in these strains. The incidence of cortisone-induced cleft palate in A/J is 100%, in B10.A, 81% and C57, 21% (ref. 4), which corresponds in rank order to the observed levels of

Table 1 Relationship of specific glucocorticoid-binding capacity to *H*-2 and genetic background

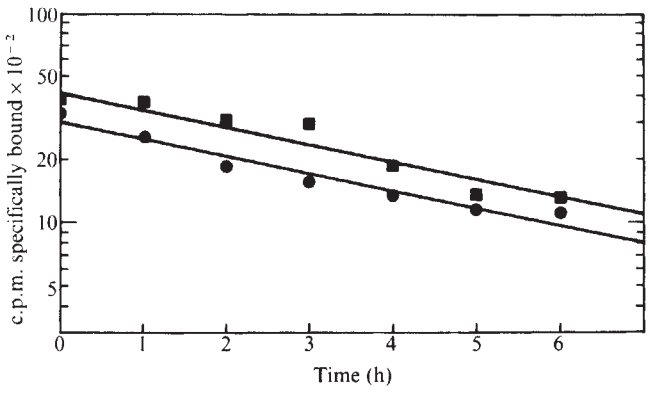
Background	Haplotype			
		H-2^a		H-2^b
A	A/J	1.29 ±0.11 (10)	A.BY	1.49 ±0.16 (9)
C57Bl	B10.A	0.99 ±0.10 (9)	C57Bl	0.83 ±0.09 (10)

Incubations (0.5 ml) containing liver cytosol and 6×10^{-8} M ^{3}H-TA in the presence of vehicle or nonradioactive competing dexamethasone were maintained for 3 h at 0 °C. Specifically bound steroid was determined as outlined in the legend to Fig. 1. The numbers represent mean specific binding (pmol of ^{3}H-TA bound per mg of cytosol protein) ± standard error for each of the four strains. Specific binding was determined in duplicate for each animal. The number of animals used to determine each mean is given in parentheses.

glucocorticoid receptors in these strains. The steroid-induced frequency of cleft palate in A.BY was not studied, but in our laboratory it has the highest spontaneous incidence of cleft palate of all four strains (19, 3, 0 and 0% for A.BY, A/J, B10.A and C57, respectively). Because A.BY also has the highest level of steroid receptors, glucocorticoid receptor levels may be an important determinant of susceptibility to spontaneous cleft palate in mice.

Although susceptibility to glucocorticoid-induced cleft palate has been shown to be partially controlled by *H*-2 haplotype, these data demonstrate that the level of hepatic glucocorticoid receptors is determined by other genetic differences between the strains. Whether the factors controlling the levels of binding activity in liver reflect those that operate in the mesenchymal cells of the fetal palate is of course open to question. We observe the same difference in glucocorticoid binding capacity in liver as was seen by Salomon and Pratt in cultured fetal facial mesenchyme cells. This strongly suggests that the observed genetic control of receptor levels is not tissue specific. Our data, unlike theirs, do not suggest different affinity constants for the receptors in the two strains. Differences in binding capacity between the strains could arise from differences in the rates of receptor synthesis or degradation, or as has been suggested[12-14], differences in rates of receptor activation and/or inactivation. Given the equivalent rates of inactivation presented in Fig. 2, the latter does not seem to be likely in this case.

Fig. 2 Unbound-receptor inactivation at 0 °C. Liver cytosol was incubated at 0 °C in the absence of labelled steroid for various times, after which saturating levels (6×10^{-8} M) of ^{3}H-TA were added in both the presence and absence of an excess of cold dexamethasone. Specifically bound steroid was determined as described in the legend to Fig. 1. ■, A/J; ○, C57.



Although *H*-2 is not the locus of prime importance in determining levels of hepatic glucocorticoid receptors in these strains, it may still be possible, using recombinant inbred lines, to identify specific loci which do regulate levels of glucocorticoid receptors. It may be particularly informative if lines with high levels of receptors and low susceptibility to glucocorticoid-induced cleft palate could be found.

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Effect of L-triiodothyronine on (−)^{3}H-dihydroalprenolol binding and cyclic AMP response to (−)adrenaline in cultured heart cells

MANY physiological responses of the cardiovascular system to thyroid hormone seem to reflect altered sympathetic activity. The effects of thyroid hormones on the heart are very similar to those induced by catecholamines and the possible interrelationship of these substances on myocardial function has been explained by an interaction at various levels. These include effects on altering catecholamine release from the sympathetic nervous system[1,2], and modifying plasma catecholamine concentrations[3,4], as well as tissue catecholamine content[5] and responses[6-8]. Although several investigators have attempted to define an action of thyroid hormone and its possible role in potentiating the catecholamine response, the mechanism responsible for this modification has not been clarified. We have reported previously that physiological concentrations of L-triiodothyronine (T_3) stimulate the rate of glucose metabolism in cultured heart cells prepared from newborn rats[9]. We now demonstrate here that cultured heart cells retain the characteristic of response to β-adrenergic stimulation and that this response seems to be influenced by T_3.

The heart cells were prepared as described previously[9,10]. The trypsinised cells were inoculated into 25-cm^2 plastic T-flasks (Falcon) at densities of approximately 200,000 cells cm^{-2} and then grown in Ham's medium (Gibco) supplemented with 10% fetal calf serum (FCS) at 37 °C (95% air, 5% CO_2).

Catecholamine action seems to involve activation of adenylate cyclase, which then leads to an increase in the intracellular concentration of cyclic AMP[11]. Therefore we examined the cyclic AMP response to (−)adrenaline and the inhibitory effect

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