Fig. 3 Voltage sensitivity of the slow i.p.s.p. spike afterpotential and input resistance of a C neurone bathed in $100 \mu\text{M}$ dTC. *a*, Intracellular recording of responses to a single antidromic shock followed by a short orthodromic train at different levels of polarizing current (left). At 0 pA and +40 pA the antidromic shock produced repetitive firing which was stopped by the i.p.s.p. (Control trials showed that the i.p.s.p. magnitude was unaffected by the repetitive firing.) *b*, Top, after balancing the bridge circuit of the microelectrode amplifier, a slow current ramp (such that $dV/dt < 4 \text{ mV s}^{-1}$) was passed into the cell and used to construct an I - V curve by playing the data into an x - y plotter. Bottom, the electrode was then withdrawn from the cell and the same current ramp was passed through the electrode alone. Membrane potentials were estimated in the range over which the unbalanced electrode resistance was $< 10\%$ of the total measured intracellularly. The unbalanced electrode resistance was $20 \text{ M}\Omega$. Subtracting this from the top plot gives slope resistances of $135 \text{ M}\Omega$ at 0 pA and $505 \text{ M}\Omega$ at -100 pA . *c*, A graph of peak i.p.s.p. amplitude versus membrane potential (V_m). *d*, Graph of peak action afterpotential versus membrane potential. Note that the bend in the cell's I - V relationship coincides with the potential at which the relationships in *c* and *d* deviate from linearity. Temperature = 22°C .

powerful influence of the i.p.s.p. on membrane properties suggests how monosynaptic inhibition might contribute to the integrative function of autonomic neurones.

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Recognition of H-2 domains by cytotoxic T lymphocytes

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The polymorphic major histocompatibility antigens (H-2) have a crucial role in the activation of antigen-specific T lymphocytes. Thus, H-2 antigens are not only recognized by allogeneic lymphocytes leading to generation of cytotoxic T lymphocytes (CTLs), but it has also been demonstrated that in syngeneic systems most T cells are only able to recognize foreign antigens in conjunction with their own MHC (major histocompatibility complex) antigens. This phenomenon, termed H-2 restriction¹, may be the key to our understanding of the biological function of MHC antigens. It is not clear whether recognition by T cells of H-2 on a molecular level is confined to particular domains on the H-2 molecule, nor whether the same polymorphic H-2 sites, which are characterized by antibodies, are recognized by allogeneic as well as by H-2-restricted syngeneic CTLs. Previous findings² indicate the existence of at least two major polymorphic domains on the H-2K^b molecule as defined by antibodies. Here we show the existence of CTLs with specificity for these polymorphic domains, and the preferential recognition of a particular domain by both alloreactive as well as H-2-restricted CTLs.

Recent competitive antibody-binding studies using six monoclonal antibodies against different determinants on the

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Fig. 4 K^+ dependence of the i.p.s.p. reversal potential. A plot of peak i.p.s.p. amplitude versus membrane potential. $\bullet$, In 2 mM K^+ Ringer's, the i.p.s.p. reversed at -110 mV . $\circ$, After 20 min washing in 10 mM K^+ Ringer's, the i.p.s.p. reversed at -68 mV . $\blacksquare$, After 20 min washing in 2 mM K^+ Ringer's, the reversal returned to -110 mV . Lines were drawn by eye. Assuming intracellular K^+ concentration to be constant, the shift in K^+ equilibrium potential, calculated from the Nernst equation, would be 41 mV . Altering extracellular K^+ concentration produced no obvious change in either the resting potential (-51 mV) or the I - V relationship of this cell. At the i.p.s.p. reversal potential, the input resistance of the cell was $247 \text{ M}\Omega$, after correcting for $24 \text{ M}\Omega$ of unbalanced electrode resistance. Temperature = 23°C .

Fig. 1 Antigenic determinants on the H-2K^k molecule are concentrated in two clusters. This schematic diagram of the spatial relationship of H-2K^k determinants was obtained by antibody competition studies in which the binding of six different radiolabelled monoclonal antibodies to H-2K^k-bearing cells was competed by addition of various cold antibodies in all possible permutations. The 50% inhibition values have been used as a very rough measure of the relative distance between determinants on the H-2K^k molecule. Antibodies within one cluster cross-block each other to varying degrees but do not interfere with binding to the other cluster, as described in more detail elsewhere². Biochemical evidence indicates that all six determinants are located on the same molecule.

H-2K^k molecule³ have provided information on the steric relationship of individual determinants². The six H-2K^k determinants defined by monoclonal antibodies are concentrated on the H-2K^k molecule in two spatially separated polymorphic domains, clusters A and B, which are shown schematically in Fig. 1. We have used these monoclonal antibodies to block the target antigen for allogeneic as well as H-2-restricted CTLs to assess which H-2 sites are target epitopes for CTLs. H-2K^k-specific CTLs were produced *in vitro* by activation of DBA/2 splenocytes with irradiated A/J stimulator cells. Figure 2 shows the ability of different concentrations of monoclonal anti-H-2K^k antibodies to block target cell lysis. None of the monoclonal antibodies completely inhibited cytotoxicity, whereas a mixture of these plus a polyvalent anti-H-2K^k alloantisera almost completely blocked target cell lysis. These findings indicate that single monoclonal antibody bound to a particular determinant on the H-2K^k molecule prevents the interaction of only some, but not all, CTL clones with the target molecule, suggesting the existence of different CTL clones with specificity for distinct H-2 determinants. This conclusion has been supported by target inhibition of alloreactive as well as H-2-restricted CTL clones generated in a limiting dilution system which showed that individual monoclonal antibodies block only 10–50% of CTL clones (our unpublished data).

Figure 2 clearly shows that the monoclonal antibodies fall into two distinct groups which differ with regard to their inhibitory capacity. Antibodies belonging to cluster A (see Fig. 2a, centre panel) reduce ⁵¹Cr release from 80% to ~40% of the plateau level while those of cluster B reduce cytotoxicity to ~20%. The same difference is observed when the monoclonal antibodies are used at maximal inhibitory concentration (1:50 or 1:100 dilution), the ratio of killer cells to targets being varied (see Fig. 2b). Again monoclonal antibodies to cluster B are much more efficient in target inhibition, suggesting that more CTL clones are reactive against determinants belonging to cluster B than against those belonging to cluster A.

Next, we analysed an H-2-restricted CTL system in which CTLs from H-2^k mice recognize the hapten trinitrophenyl (TNP) linked to the H-2K^k molecule⁴. CBA splenocytes were sensitized *in vitro* with TNP-conjugated CBA spleen cells and cytotoxic activity was determined after 6 days of culture on TNP-conjugated H-2^k target cells. Control experiments established that the TNP-specific CTLs were predominantly restricted by the K end (data not shown). Inhibition of the TNP-specific CTLs by anti-K^k antibodies showed again that each individual antibody blocks only a fraction of the CTL effector population while a mixture of monoclonal antibodies or a polyvalent antiserum against K^k almost completely prevents lysis (see Fig. 3). These findings indicate that CTLs can recognize TNP in conjunction with different H-2 determinants, or in other

words, that one H-2K^k molecule carries more than one restriction site. As was also observed for allogeneic anti-K^k CTLs (Fig. 2), it was found for the TNP system that antibodies to cluster B were much more effective in preventing target cell lysis than antibodies to cluster A. The same conclusion was reached when the antibody concentration was constant at maximal inhibitory concentration (1:50–1:100 dilution) and the killer cells titrated (data not shown).

Calculation of lytic units provides an estimate of the relative number of CTLs in a population⁵. These data (see Table 1) show that in the presence of monoclonal antibodies to cluster B, on average three times fewer lytic units are measured than in the presence of monoclonal antibodies to cluster A. Taken together, these data suggest that about three times as many alloreactive and self-restricted CTLs are present which react with determinants of cluster B.

Target inhibition by monoclonal antibodies has also been described by others^{6,7} but our report provides additional information on the correlation of CTL specificity with the steric arrangement of H-2 determinants. The data show that both alloreactive as well as TNP-specific H-2-restricted populations are heterogeneous with regard to which H-2 epitopes on a given molecule are recognized, and that both CTL populations show preference for a particular domain on the H-2K^k molecule which is characterized by antibodies to cluster B. It is not clear from our results whether CTLs recognize exactly the same determinants as monoclonal antibodies or whether the latter block spatially related determinants only by steric hindrance. Another possibility is that monoclonal antibodies induce con-

Fig. 2 Target inhibition of H-2K^k-specific alloreactive CTLs by monoclonal anti-H-2K^k antibodies. 10×10^6 DBA/2 (d/d) spleen cells were stimulated with 12×10^6 A/J (k/d) irradiated spleen cells as described elsewhere⁹. After 5 days, CTL activity was measured against ⁵¹Cr-labelled L929 (H-2^k) target cells for 4 h in the presence of anti-K^k antibodies in a total volume of 200 μ l using methods described previously⁶. These CTLs were shown to be specific for K^k (results not shown). In some experiments B10.D2 responders and B10.A stimulators were used. The figure shows a typical experiment which was repeated several times with the same results. **a**, Effect of different concentrations of monoclonal antibodies ascites on cytotoxicity at an effector to target ratio of 50:1. Left frame: $\circ$, medium control; Δ , control ascites 13/18; $\square$, hyperimmune BALB/c anti-A/J alloantisera; $\bullet$, mixture of six monoclonal anti-K^k antibodies. Middle frame, anti-K^k antibody describing cluster A: ∇ , m3 = H100-5R28; $\blacksquare$, m4 = H100-27R55; $\blacklozenge$, m1 = H116-22R7. Right frame, monoclonal anti-K^k antibody describing cluster B: $\diamond$, m5 = H100-30R23; ∇ , m9 = H142-23/3; $\blacktriangle$, m10 = H142-45/2. **b**, CTL titration in the presence of a constant amount of blocking antibodies (1:50 dilution of ascites). Same symbols as for **a**.

Fig. 3 Target inhibition of TNP-specific H-2K^k-restricted CTLs from CBA (H-2^k) mice by monoclonal anti-K^k antibody. TNP-specific CTLs were produced *in vitro* by activation of CBA splenocytes against TNP-conjugated CBA spleen as described elsewhere⁴. CTL activity was measured at an effector to target ratio of 50:1 against TNP-conjugated ⁵¹Cr-labelled L929 (H-2^k) target cells in the presence of various concentrations of blocking antibodies. By using different targets as well as cold target inhibition it was established that most CTLs were restricted by K^k. From other studies it is known that the antibodies used here mediate their blocking effect by inhibition of the target determinant and not when they are directed against the effector cells⁶. Symbols are the same as for Fig. 2. Left frame: ○, medium control; △, control ascites 13/18; □, BALB/c anti-A/J alloantisera; ●, mixture of six monoclonal anti-K^k antibodies. Middle frame, ascites of monoclonal antibody to cluster A (m1, m3, m4); right frame, monoclonal antibody to cluster B (m5, m9, m10).

formational changes at distal parts of the H-2 molecule which influence the interaction with CTLs. However, we consider it more likely that both CTLs and antibodies recognize basically the same polymorphic domains. The different blocking capacity of monoclonal antibodies to clusters A and B is not likely to be due to distinct physical properties of the monoclonal antibodies because those used here have similar affinities (ref. 8 and H. Lemke, personal communication) and belong to similar immunoglobulin classes (IgG2a and IgG2b, respectively). An exception is H145-45/2, which after recent re-evaluation was found to be IgM (unpublished data); the fact that it is pentameric may explain its very strong inhibitory capacity.

A possible explanation for the striking preference of alloreactive and H-2-restricted CTLs for cluster B is that both H-2^d (DBA/2 or B10.D2) and H-2^k mice use a similar or related pool of T-cell receptors for recognition of the K^k molecule, whether an alloantigen (in the case of DBA/2) or a self antigen (in the case of CBA) is recognized. Such a hypothesis would be compatible with dual recognition models, one binding site being for TNP and the other for H-2 determinants. Arguments in favour of single recognition models assume that neodeterminants formed by TNP modification of either H-2 or other surface components are predominantly located in the

direct vicinity of polymorphic H-2 domains. Target inhibition of restricted CTLs by anti-H-2 would then be caused by steric hindrance. Note that preference of different CTL populations for the same cluster could be merely coincidental as it is possible that an H-2 molecule carries only few regions which, for steric reasons, are accessible to T cells. Further analysis of K^k-restricted CTL systems will clarify this question.

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Light chain isotypes selectively associate with heavy chain idiotypes in T-dependent and T-independent dextran-specific precursors

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In murine immunoglobulins, the κ chain is the major light chain isotype, as exemplified by the small amount of λ immunoglobulins present in normal sera, the rare occurrence of λ plasmacytomas and the preponderance of κ light chains in induced antibodies. Notable exceptions to this are the response to $\alpha(1 \rightarrow 3)$ dextran¹ in BALB/c mice and the heterocytic antibodies raised against the nitrophenol hapten in C57BL/6 mice². In the former response, the expression of idiotype in the hypervariable regions of the heavy chain³ is characteristically restricted. We have now analysed the B-cell precursors for the $\alpha(1 \rightarrow 3)$ linkage-specific response induced by T-dependent and T-independent dextran antigens with respect to light chain isotype and heavy chain idiomorph expression. Our findings clearly demonstrate that the two forms of dextran antigen trigger different B-cell precursor subpopulations. Furthermore, each individual animal appears to possess a different repertoire of idiomorph-committed precursors. This contrasts with the response of BALB/c mice to phosphorylcholine (PC) where most anti-PC antibodies and precursors respond with the dominant expression of the T15 idiomorph family, regardless of whether T-dependent or T-independent PC antigens are used⁴.

BALB/c mice immunized with the T-independent antigen dextran B1355s respond with the expression of unique idiotype (IdI) J558 and M104E, and a cross-reacting IdX idiomorph⁵. Schilling and co-workers⁶ have shown that the unique IdI specificities are generated during the V-D-J joining process of heavy chain genes. While the expression of λ chains in the T-independent anti-dextran BALB/c response is consistently >90%, the amount of J558 and M104E IdI idiotype varies between individual animals^{7,8}. This finding led us to speculate that the variable expression of heavy chain idiotype might be caused by individually different, idiomorph-specific regulation of B-cell precursors. Alternatively, during the development of the dextran-specific repertoire individually different exposures to environmental antigens could influence precursor maturation. According to the latter hypothesis, serum expression of light

Table 1 Lytic units of alloreactive and self-restricted CTL effector cells in the presence of blocking antibody against target determinants

Anti-H-2 antibody added to effector phase	H-2 cluster recognized	Source of CTLs	
		DBA $\bar{a}$ A/J	CBA $\bar{a}$ CBA-TNP
No antibody	—	10.0	14.1
13/18 (Ia.7)	—	10.0	13.2
H100-5R28 (m3)	A	1.03	2.6
H100-27R55 (m4)	A	0.84	1.9
H116-22R7 (m1)	A	1.33	4.35
H142-23/3 (m9)	B	0.35	0.54
H100-30R23 (m5)	B	0.35	0.82
H142-45/2 (m10)	B	0.12	0.72
Mixed monoclonal antibodies	A+B	0.02	<0.01

Lytic units (LU) were calculated according to Cerottini *et al.*⁵ on the basis of CTL effector titrations as shown in Fig. 2b for alloreactive CTLs. The corresponding titration curves for TNP-specific CTLs are not shown. One lytic unit is defined as the number of effector cells required to lyse 50% of the target cells.