potential mechanism of oncogene action. p185 has many characteristics similar to those of the receptors for epidermal growth factor (EGF)[19] and platelet-derived growth factor (PDGF)[20], including approximate molecular weight, plasma membrane localization and phosphorylation. p185 is also a glycoprotein (D.F.S., unpublished results), as are the EGF and PDGF receptors. Waterfield and colleagues have presented persuasive evidence that the viral *erb-B* oncogene represents a truncated EGF receptor gene which lacks the extracellular EGF-binding domain but nonetheless delivers to the cell a mitogenic signal which results in neoplastic transformation[21]. The *neu* gene product p185 may also be an altered growth factor receptor which transforms cells by a mechanism similar to that of v-*erb-B*. Its localization to the cell surface and association with oncogene activation suggest that p185 may prove useful as a potential target for directing monoclonal antibodies against tumours containing activated *neu* oncogenes; we are presently investigating this possibility.

We thank Mr Jim Throp for preparation of the manuscript, Willis Chung for technical assistance in the early phases of this study, and Drs Ann Carroll, Adam Lowy and John Monroe for thoughtful criticism. J.A.D. was supported by NIH grant 5-T32-GM07753 from the Medical Scientist Training Program at Harvard Medical School. D.F.S. was supported by postdoctoral fellowships from the Damon Runyon Cancer Fund and the NIH. This work was supported by grant CA 14723 from the NCI and by an American Cancer Society Faculty Award to M.I.G.

Received 18 June; accepted 19 September 1984.

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Therapy with monoclonal antibodies by elimination of T-cell subsets *in vivo*

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A major aim in immunology has been to understand how the immune system evokes characteristic responses to infection, foreign tissue grafts and tumours. The current view of immunoregulation is based mainly on studies of lymphocyte subsets, either *in vitro*[1-3] or by adoptive transfer to irradiated recipients[4]. Many reagents are available for defining T-cell subsets[5-8], but only recently have there been helper T-cell-specific antibodies[9,10] against the mouse equivalent of the Leu3/T4 (man) and W3/25 (rat) antigens. It is clear that monoclonal antibodies[11] will eventually replace anti-lymphocyte globulin for immunosuppression in organ grafting[12,13], but although there has been some clinical success[14-16], most monoclonal reagents cause only transient reductions in their target cells *in vivo*[17-20]. This uncertainty in the potency of monoclonal antibodies has led some workers to consider them as targeting agents for such highly cytotoxic drugs as ricin A (ref. 21). We show here that unmodified monoclonal antibodies can be extremely effective at depleting cells *in vivo* and can be used for the selective manipulation of different aspects of the immune response.

The observation that two different rat IgG2b anti-mouse Thy-1 antibodies were potently immunosuppressive when administered *in vivo*, compared with a range of other monoclonal antibodies of the same specificity but different isotypes[22,23], suggested that the antibody isotype might be an important factor for serotherapy. We therefore prepared[24] rat monoclonal antibodies to several mouse T-cell antigens using the Y3/Ag1.2.3 myeloma[25] as fusion partner and compared these for their ability to suppress the *in vivo* antibody response to sheep red blood cells (Fig. 1). Adult thymectomized mice were used so that new T cells would not be produced after monoclonal antibody depletion, and immunosuppression could then be measured in the absence of any short-term effects of the antibody. Only the IgG2b antibodies recognizing an antigen expressed on the helper T cells (Thy-1, Lyt-1 and L3/T4) were effective at eliminating the antibody response. Six rat anti-Thy-1 monoclonal antibodies of other isotypes (IgM, IgG2a and IgG2c) and one IgG2a

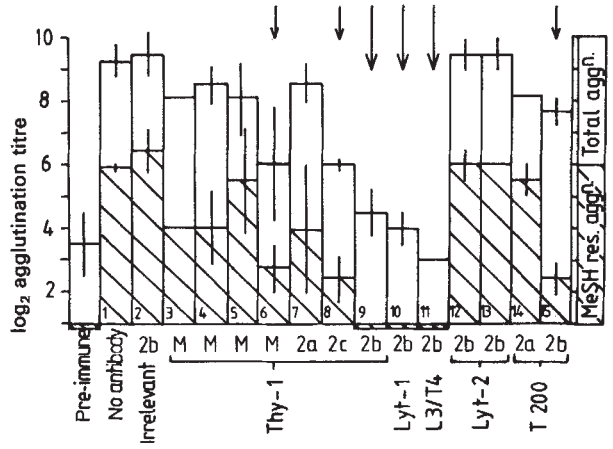

Fig. 1 Suppression of the antibody response to SRBCs. Male CBA mice were thymectomized at 5-6 weeks of age and randomly selected 3 weeks later for monoclonal antibody administration, given as two intravenous injections, 1 week apart, of 0.2 ml of monoclonal ascitic fluid [from $(LOU \times DA)F_1$ rats], equivalent to ~0.5 mg of active antibody in the following groups: 1, no antibody ($n = 10$); 2, irrelevant IgG2b ($n = 2$, YTH 89.1 anti-human glycophorin A; G. Hale, personal communication); 3-6, four different IgM anti-mouse Thy-1[23] (YTS 27.3, $n = 2$; YTS 109.8, $n = 2$; YTS 198.3, $n = 2$; YTS 148.3, $n = 4$); 7-9, IgG2a(YTS 111.4, $n = 2$), IgG2c(YBM 29.2, $n = 4$), IgG2b(YTS 154.7, $n = 4$) anti-mouse Thy-1[23]; 10, IgG2b anti-mouse Lyt-1 ($n = 4$; YTS 121.5 which cross-inhibits 53-7.313 (ref. 7 and our unpublished observation); 11, IgG2b anti-mouse L3/T4 [$n = 4$; YTS 191.1 which is present on only the Lyt-2 negative peripheral T cells (Table 1), 85-90% of thymocytes and <2% of bone marrow cells, reacts with a protein of relative molecular mass 55,000 as determined by 'Western blots' (ref. 45 and our unpublished observations) and is therefore similar to GK1.5 (ref. 10); 12, 13, two IgG2b anti-Lyt-2 [YTS 169.4, $n = 6$; YTS 172.3, $n = 6$, which cross-inhibit 53-6.72 (ref. 7 and our unpublished observation]; 14, 15, IgG2a(YBM 42.2 (ref. 46), $n = 2$) and IgG2b(YW 62.3, $n = 2$, which cross-inhibits YBM 42.2, our unpublished observation) anti-T200 ('leukocyte common antigen'[47]). Animals were given 0.2 ml of 10% SRBCs intraperitoneally 3 weeks after monoclonal antibody (except for preimmune group, $n = 6$). These were bled from the tail vein after 10 days and the serum agglutination (agg^n.) titre determined with SRBCs alone (total IgM and IgG response) or with SRBCs in the presence of 0.1 M 2-mercaptoethanol (MeSH-resistant IgG response). Substantial immunosuppression is indicated by the arrows.

anti-T200 were far less effective than the IgG2b reagents of the same specificities.

The observed immunosuppression could be accounted for by depletion of helper or total T cells (Table 1). At various times from 1 week to 3 months after the original injection of monoclonal antibody, groups of treated, or control thymectomized, mice were compared for the expression of various T-cell markers using the cytofluorograph. All of the rat IgG2b antibodies gave substantial and long-term depletion (5 weeks post-antibody in Table 1) of their target T cells. Residual rat immunoglobulin could not be detected and an anti-globulin response was observed by day 10 (in all but YTS 191.1-treated mice, results not shown). This means that suppression was unlikely to be due to blocking of function, temporary changes in the pattern of lymphocyte circulation or 'antigenic competition'[26], which could contribute to the short-term effects described in other systems[23,27], such as that of LeGros *et al.*[28].

Selective manipulation of the major T-cell subsets could be readily achieved with the IgG2b monoclonal anti-L3/T4 or -Lyt-2 antibodies (Table 1). For example, if we consider the YTS 169.4 (anti-Lyt-2) treated group, the Lyt-2-postive cells were reduced to 0.2%, which represents a specific depletion of at least 96% compared with thymectomized controls. Elimination of cells was also effective in the lymph-nodes, with 14.5% Lyt-2-positive T cells in controls compared with 1.2% in treated mice (results not shown). The loss of Thy-1, L3/T4 and Lyt-1 in these treated mice was consistent with a reduction in only the Lyt-2-positive T cells. Over a 12-month observation period, there was no observable change in Lyt-2 expression. This demonstrates that any remaining Lyt-2-positive T cells were unable to repopulate the animal and that, in these conditions, the mature Lyt-2-negative phenotype was stable and irreversible, in contrast to some suggestions of phenotypic switching[29]. Similar long-term depletions were observed in the YTS 191.1 (anti-L3/T4) treated mice, although we have not followed these animals beyond 6 months post-injection. It is possible that further injections of antibodies are required to maintain depletion during a very strong antigenic challenge, where small numbers of residual T cells might be induced to proliferate.

Immunological responses in T-cell subset-depleted mice were those expected considering the surface phenotype of the remaining T cells. Mice depleted of L3/T4-positive cells did not make an antibody response to sheep cells (Fig. 1) or the injected rat immunoglobulin (results not shown), and were also unable to develop delayed hypersensitivity (HSV-DH, Table 1) to herpes simplex virus (recently implicated as an important effector mechanism in immunity to such viruses[30] and in allograft rejection[31]). Mice depleted of either subset (L3/T4 or Lyt-2) were unable to produce cytotoxic T cells to allogeneic targets *in vitro* (Table 1, Fig. 2). This is because the generation of cytotoxic T cells depends on the presence of both helper ($Lyt\text{-}2^-$) and cytotoxic precursor ($Lyt\text{-}2^+$) cells[32]; simple mixing of the cells from the L3/T4- and Lyt-2-depleted animals at the start of the culture period reconstituted the response (Fig. 2). Therefore, animals can be independently depleted of the 'helper' or 'cytotoxic' T-cell lineages by administering monoclonal antibodies to L3/T4 and Lyt-2. These animals have remained functionally depleted for at least 6 months after monoclonal antibody injection. Previously, this could only be achieved, albeit inefficiently, by using populations of T cells depleted of $Lyt\text{-}1^+$ or $Lyt\text{-}2^+$ subsets *in vitro* to reconstitute lethally irradiated, thymectomized mice[4]. Such animals may have a contribution from radio-resistant T cells[33], and complications associated with irradiation are avoided by the *in vivo* antibody treatment. The major advantage of using subset-specific monoclonal antibodies directly in animal models is that it should be possible to apply the rules learned in, for example controlling allograft rejection, to therapy in man.

It is still not entirely clear which T cells are responsible for the rejection of skin grafts in mice[31,34,35], partly perhaps because of some confusion over Lyt-1 expression[33]. We used CBA mice depleted of either L3/T4- or Lyt-2-positive T cells to determine the cells required to reject fully mismatched (BALB/c) or H-2-matched (minor antigens mismatched, B10.BR) skin grafts (Fig. 3). In both cases, Lyt-2 depletion did not delay rejection, while L3/T4 depletion led to greatly increased survival of the grafts. These results were confirmed using normal CBA mice (with a thymus) given a 14-day course of antibody to cover the initial

Table 1 Depletion of T-cell subsets *in vivo* by serotherapy with monoclonal antibodies

Antibody treatment	% Positive spleen cells: Thy-1 (154.7)	Lyt-1 (121.5)	Lyt-2 (169.4)	L3/T4 (191.1)	L3/T4 : Lyt-2 ratio	% Specific depletion	HSV-DH ($\times 10^{-2}$ mm)	Allo-CTL (% control)
YTS 154.7	<u>1.6</u>(−)	2.3(+)	0.6(−)	ND	ND	90	10.4 ± 1.5	ND
YTS 121.5	2.5	<u>2.7</u>(−)	0.7(−)	ND	ND	82	5.5 ± 1.0	21
YTS 169.4	10.3	11.0	<u>0.2</u>(−)	11.3	57	96	13.0 ± 1.8	2
YTS 191.1	8.8	4.7	5.0	<u>1.5</u>	0.3	86	4.5 ± 2.0	0
None (ATX)	15.7	14.6	5.1	10.8	2.1	0	15.4 ± 2.3	100
Normal	33.5	35.0	11.4*	24.3*	2.1	ND	ND	ND

Male CBA mice were thymectomized at 5–6 weeks of age, randomly selected for injection of monoclonal antibodies and tested 3 weeks later for their responses to sheep red blood cells (SRBCs) as described in Fig. 1 legend, HSV-specific delayed hypersensitivity (DH) as described below or generation of allo-CTL as described in Fig. 2. Two mice from each of the SRBC-primed group were selected at random, 5 weeks after monoclonal antibody injection, and the spleen (and mesenteric lymph nodes, data not shown) was taken for analysis of T-cell markers. Red cells were lysed by NH_4Cl treatment and the remaining lymphocytes incubated for 1 h at 4 °C in antibody supernatants at 100 μg ml^{-1} of medium containing 5% heat-inactivated normal rabbit serum to block Fc binding, and 0.1% NaN_3. After washing, bound monoclonal antibody was detected by incubation with fluorescein isothiocyanate (FITC)-labelled mouse monoclonal antibodies to rat immunoglobulin light chains (MAR 18.5)[42] and rat IgG2b (NORIG 7.16)[36]. Cells were washed in medium containing 50 μg ml^{-1} propidium iodide to stain dead cells, and resuspended in phenol red free medium containing 0.5% bovine serum albumin and 0.1% NaN_3. Samples were analysed using the H50 Cytofluorograf with 2150 computer (Ortho Diagnostic) at 1,000 cells s^{-1}. Computer gating was used on forward and 90° light scatters, and on red fluorescence (propidium iodide) such that histograms of green (fluorescein) fluorescence were obtained for live, monomeric cells. Numbers show % positive cells (above fluorescence channel 40 on a linear scale of 1,000 channels: controls with the FITC-labelled anti-globulins alone gave background of 0.1–0.3% positive) and are means of the results obtained from two mice in each group analysed individually. % Specific depletion is the % depletion of cells with the phenotype of injected antibody specificity as compared with ATX controls. Also shown for comparison are results for two normal (not thymectomized) CBA mice. HSV-DH: For the induction of virus-specific delayed hypersensitivity, 10^5 plaque-forming units (PFU) HSV-1 TK^- (strain CL101 deficient in thymidine kinase) was injected into the ear pinna, as previously described[43]. Seven days later the mice were challenged with 5×10^5 PFU HSV-1 TK^- in the contralateral pinna and the DH response was measured 24 h later as the increase in ear thickness[44]. The results are expressed as the arithmetic mean ± s.d. of 5 mice per group. CBA mice receiving only the challenge inoculum, as a control for nonspecific inflammation, gave $3.8 \pm 1.2 \times 10^{-2}$ mm. Allo-CTL: % control cytotoxic T lymphocytes calculated from the effector : target ratio required to give 30% specific lysis (Fig. 2). Plus or minus in parentheses indicates respectively a ~10% increase ~or a 25–50% decrease in mean fluorescence of positive cells. ND, not determined. Underlining emphasizes the depleted population.

* Lyt-2 + L3/T4 gave 38.7% positive.

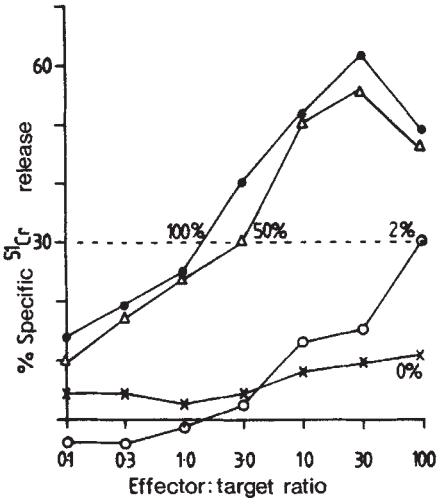

Fig. 2 L3/T4- and Lyt-2-negative mice are independently depleted of the helper and cytotoxic T-cell lineages. ●, Normal thymectomized (ATX) mice; ×, YTS 191.1 (anti-L3/T4) treated ATX mice; ○, YTS 169.4 (anti-Lyt-2) treated ATX mice; △, 50:50 mix of cells at start of culture from YTS 191.1 and YTS 169.4 ATX mice. **Methods:** Spleens were taken from two normal, two YTS 191.1 (anti-L3/T4), and two YTS 169.4 (anti-Lyt-2) treated, adult thymectomized male CBA mice, 3 weeks after monoclonal antibody administration. Responder cells from individual mice were incubated at both 2×10^6 and 5×10^6 cells ml^{-1} with 10^6 irradiated (2,500 rad) BALB/c stimulator spleen cells in 1-ml Linbro cultures, in Iscovés modified Dulbecco's medium containing 5% fetal calf serum (FCS) at 37 °C for 6 days with constant rocking. Effector cells were collected and pooled from the mixed lymphocyte cultures for each animal and titrated (in triplicate) in 100-μl microtitre wells with 10^4 BALB/c spleen cells that had been previously stimulated with concanavalin A for 2 days before labelling with ^{51}Cr-labelled sodium chromate. The supernatants were removed for γ counting after 4 h at 37 °C. Cytotoxic activity was measured as the % specific ^{51}Cr release, calculated as:

$$\%\ \text{Specific release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100$$

where the spontaneous release was from labelled targets alone, and the total release was from detergent-treated targets. Effector: target ratios were calculated on the basis of the number of cells added at the start of the mixed lymphocyte culture. Each point is the mean of two experiments. Approximate % of control activity as shown was calculated from the effector to target ratio required to give 30% specific release.

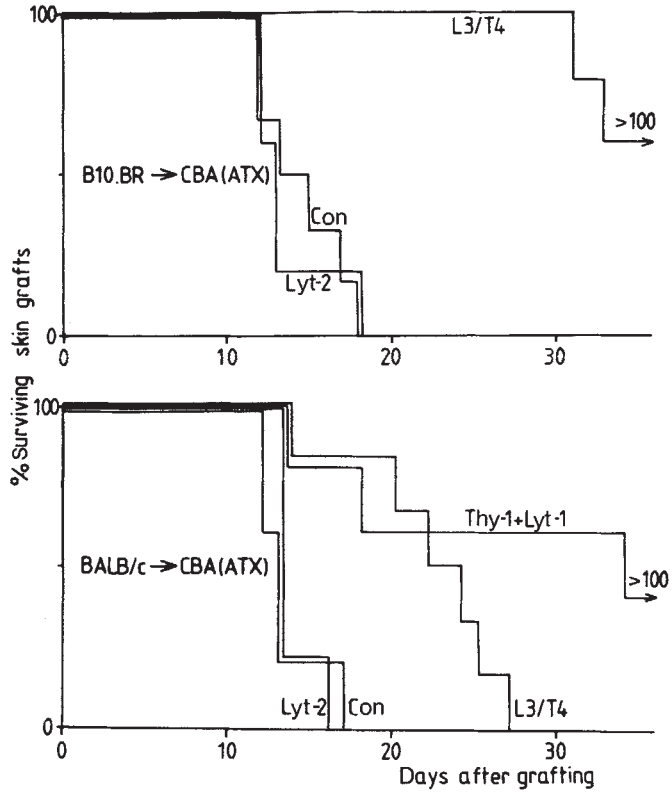

Fig. 3 Suppression of skin allograft rejection with monoclonal antibodies. Per cent surviving grafts are shown for the following groups: Con, controls with no antibody treatment; Lyt-2, YTS 169.4 (anti-Lyt-2) treated group; L3/T4, YTS 191.1 (anti-L3/T4) treated group; Thy-1 + Lyt-1, YTS 154.7 + YTS 121.5-treated group. Groups of 4–10 thymectomized CBA mice were given 2×0.2 ml of rat IgG2b monoclonal antibodies intravenously as described in Fig. 1 legend. BALB/c differing in H-2 and minor histocompatibility antigens or B10.BR (minor histocompatibility differences) tail skin was grafted to the lateral thoracic wall 4 weeks and 8 weeks after antibody treatment respectively, and covered with Vaseline-impregnated gauze and plaster bandage. The dressings were removed on day 10 and the grafts inspected every day for rejection.

period of grafting with fully allogeneic (BALB/c) skin (mean graft survival in days: no antibody, 11±2; anti-Lyt-2, 12±2; anti-L3/T4, 25±3; anti-Thy-1, 25±3). Immunoperoxidase staining[36] of spleen sections taken on the day after rejection confirmed that the target cells were still relatively depleted. These results suggest that T cells with the phenotype Thy-1$^+$, L3/T4$^+$, Lyt-2$^-$ are the major promoters of primary graft rejection in mice. However, Lyt-2-positive cells may have a secondary role, particularly in fully mismatched grafts, where removal of all T cells was more effective than removing just the L3/T4 cells (Fig. 3) and anti-Lyt-2 injection from day 7 of grafting in previously untreated mice did lead to delayed rejection (mean survival 18±3 days compared with 13±2 days in untreated controls, not shown). This confirms and extends the results obtained by adoptive transfer into lethally irradiated, thymectomized rats where the W3/25$^+$ helper T cells were sufficient to cause graft rejection[37].

The above results demonstrate that selected rat IgG2b monoclonal antibodies can be very effective *in vivo*. These would also seem to be the best candidates for human therapy[38], because rat IgG2b is the only subclass known both to fix human complement[39,40] and to activate antibody-dependent cell-mediated cytotoxicity *in vitro*[41], the latter even at very low antibody concentrations. Although most of the above experiments used large (~0.5 mg) amounts of antibody, YTS 169.4 is able to deplete Lyt-2 cells, and YTS 191.1 can suppress antibody responses using a total of 1–10 μg per thymectomized mouse.

Using specific monoclonal antibodies to manipulate the immune response, there are at least two ways in which fundamental aspects of immunoregulation can be investigated. First, subset-depleted mice can be used to determine the T cells involved in different aspects of bacterial or viral infections. Such animals are also a source of subset-depleted cells for *in vitro* or adoptive transfer work, with the advantage that no antibody or complement need be present at the time of the experiments. Second, T-cell subsets can be manipulated at any time during a response to foreign antigen or in autoimmune diseases, in order to probe the events which regulate the immune system as a whole. Elimination of cells *in vivo* is also a requirement for the treatment of neoplastic disease, and the above reagents are being used to develop a model for the serotherapy of leukaemias. The use of monoclonal antibodies as therapeutic agents in animal models should allow us to extend strategies for serotherapy which are directly applicable to man.

We thank Gill Martin, Penny Lovat and Jenette Phelan for their technical assistance. This work was supported by MRC grants.

Note added in proof: We have recently shown that Lyt-2$^+$ cells may have a major role in graft rejection when the animals have been previously primed to the graft antigens.

Received 5 June; accepted 10 September 1984.

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Dynorphin(1–13) improves survival in cats with focal cerebral ischaemia

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Since the discovery of opiate receptors in the central nervous system (CNS)[1–3], it has become apparent that endogenous opiate ligands are involved in CNS function. Most attention has focused on their role in modulating pain, but they have also been implicated in various physiological functions and in disease states. We are concerned with evidence that endogenous opioid peptides may also contribute to the neurological deficits arising from cerebral ischaemia[4,5]. Dynorphin, which is widely distributed in the brain and pituitary[6,7], has been reported to produce unusual motor and behavioural effects[8–11] and may act as a regulatory neuropeptide, not as a classical opiate agonist or antagonist[12–15]. We have therefore administered to cats in which the right middle cerebral artery had been occluded both dynorphin (1–13) and analogue and control materials. We find that dynorphin (1–13) prolongs survival.

Forty-two adult male cats (3-5 kg) assigned randomly to one of four groups were anaesthetized with halothane and subjected transorbital occlusion of the right middle cerebral artery[16]. A subcutaneous pocket was created for later placement of an osmotic pump through a small midline lumbar incision, and the cat allowed to awaken. Six hours after occlusion, sensory and motor function, consciousness and pupillary reaction to light were graded on an 18-point scale (Table 1) by two persons who did not know to which group the cat had been assigned. The cats then received an intraperitoneal injection (2 ml) of one of the following solutions: (1) sterile normal saline; (2) Leu-enkephalin, 750 $\mu g\,kg^{-1}$ (1.35 $\mu mol\,kg^{-1}$) in saline; (3) dynorphin(1-13), 2 $mg\,kg^{-1}$ (1.25 $\mu mol\,kg^{-1}$) in saline; or (4) dynorphin(3-13), 2 $mg\,kg^{-1}$ (1.43 $\mu mol\,kg^{-1}$) in saline. Neurological assessment was repeated 20 min after injection.

Table 1 The 18-point scale used for assessing neurological function in cats examined 6 h after middle cerebral artery occlusion

Neurological response	Score
Motor function	
Cat walks with normal gait—no neurological deficit	6
Cat walks with abnormal gait, has mild hemiparesis	5
Cat barely walks with moderate hemiparesis	4
Cat unable to walk with moderate hemiparesis	3
Cat unable to walk with severe hemiparesis	2
Cat unable to walk with hemiplegia	1
Sensory function	
Cat responds appropriately to tactile and noxious stimuli	5
Cat responds appropriately to noxious limb stimuli only	4
Inappropriate response to noxious limb stimulation only	3
Reflex response to noxious limb stimulation only	2
No response to noxious limb stimulation	1
Level of consciousness	
Awake and alert	5
Awake and alert with lack of spontaneous movements	4
Drowsy, responds only to noxious stimuli	3
Stuporous, minimal response to noxious stimuli	2
Comatose	1
Pupillary response	
Unilaterally reactive to light	2
Unilaterally unreactive to light	1

Under ketamine sedation, an osmotic pump (Alzet 2MLI, Alza Corporation) was implanted in the subcutaneous pocket to deliver saline at 10 $\mu l\,h^{-1}$, Leu-enkephalin at 20 $\mu g\,h^{-1}$ (36 $nmol\,h^{-1}$), dynorphin (1-13) at 50 $\mu g\,h^{-1}$ (31 $nmol\,h^{-1}$), or dynorphin (3-13) at 50 $\mu g\,h^{-1}$ (36 $nmole\,h^{-1}$). The pumps were coded so that each cat received the drug with which it had been injected earlier.

The cats were housed in metabolic cages at constant temperature and humidity and were examined frequently. Penicillin G, 1 MU per day, was administered intramuscularly; lactated Ringer's solution was injected subcutaneously to provide adequate daily fluid maintenance until the cat began to eat and drink. So long as the cats were alive, their neurological condition was assessed daily by the battery of tests shown in Table 1; 7 days after occlusion all the cats were killed. The brain was removed and a coronal section made at the optic chiasm. The slices were incubated in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 25 min[18,19]. Colour slides were made of the stained slices, and the affected hemisphere and infarcted area were traced from projected images of the slide. Using a digitizing pad (BIT PAD ONE, Summagraphics, Fairchild, Connecticut) and a planimetry program on a PDP-11/23 computer, infarct size was calculated as the percentage of infacted tissue relative to the entire hemisphere.

Neurological scores before and 20 min after acute injection of the peptides were compared by non-parametric analysis of variance (Kruskal-Wallis) followed by *post hoc* individual comparison, and the infarct sizes were compared by one-way analysis of variance. Kaplan-Meier survival curves were constructed and Gehan's statistics used to compare the survival between the groups.