

INHIBITION OF ALLOGRAFT AND CONCORDANTXENOGRAFT REJECTION

This invention relates to pharmaceutical formulations useful in the treatment of chronic rejection of allografts and rejection of concordant xenografts. The invention therefore relates to the promotion of graft survival, or prevention of graft loss, in a host.

Allografting is the transplantation of tissue between two members of the same species. Xenografting, which is the transplantation of tissues between two different species, has been divided by biological criteria into two subsets (Calne, *Transplant. Proc.* II 550-556 (1970)). The two subsets are concordant xenografts and discordant xenografts. The distinction between these two subsets is that a vascularised organ graft transplanted between discordant species is rejected hyperacutely, that is to say within a few minutes or hours of revascularisation. Between concordant species, hyperacute rejection of xenografts does not take place. Grafts will, however, be rejected, and often in an accelerated manner, but this will occur over several days and not within hours or minutes.

It is a generally held belief, although formal evidence for this is lacking, that between discordant species hyperacute rejection is a result of the action of naturally occurring antibodies against the donor species. Concordant grafts on the other hand survive at least for a short while because no such antibodies capable of causing their destruction are present. There are many examples in the literature to

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1 demonstrate this difference. Perhaps the most elegant
2 are those of Hammer (in "Xenograft 25" (Mark A. Hardy,
3 Ed) Elsevier, Amsterdam (1989)). Using always the dog
4 as the recipient species, Hammer indicates the survival
5 times of kidney grafts to be as follows:

6		
7	Dog-Dog	11.4 days
8	Dingo-dog	11.5 days
9	Wolf-dog	19.4 days
10	Fox-dog	6.5 days
11	Lion-dog	10-24 hours
12	Tiger-dog	10-24 hours
13	Cat-dog	10-24 hours
14	Pig-dog	10-30 minutes

15
16 The last four interactions would on this basis be
17 discordant xenografts, whereas the first four
18 xenografts would be concordant. Hammer has equated
19 these differences between discordant and concordant
20 xenografts to the presence or absence, respectively, of
21 cross-species haemagglutinating antibodies.

22
23 At an academic level, there are some isolated
24 difficulties with what is essentially a working
25 definition of concordant and discordant species
26 relationships. As a practical matter, however, for the
27 purposes of the present invention, two different
28 species are taken to be related in a concordant fashion
29 if there is no hyperacute rejection of a xenograft from
30 one to the other. For the purposes of the present
31 invention, therefore, species are concordantly related
32 if, even though they would normally give rise to
33 hyperacute rejection, they have nevertheless been

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1 subjected to a procedure, for example genetic
2 manipulation, so as to prevent hyperacute rejection.
3 International Patent Application PCT/GB90/01575 for
4 example relates in specific embodiments to potential
5 xenograft donor animals which have been transgenically
6 modified to express recipient complement down
7 regulators (such as decay accelerating factor (DAF) or
8 membrane cofactor protein (MCP)). Organs from the
9 donor animals are not hyperacutely rejected when
10 grafted onto an animal of what would normally be
11 regarded as a discordantly related species. For the
12 purposes of this invention, therefore, such transgenic
13 donor animals may be regarded as concordantly related
14 to their intended target species.

15

16 Examples of species having a concordant relationship,
17 as defined above, include hare-rabbit, hamster-rat and
18 non-human primate-human, including, for example,
19 baboon-human, chimpanzee-human and macaque-human.

20

21 The promotion of graft survival has received attention
22 both in the field of xenografts (discordant and
23 concordant) and allografts. Taking first suggestions
24 that have been made for the promotion of discordant
25 xenograft survival, Adachi et al (*Transplant. Proc.* XIX
26 (1) 1149-1152 (1987)) have studied the survival of
27 discordant cardiac xenografts in rats. Among the
28 potential survival promoters studied were cyclosporin
29 A, aspirin and cobra venom factor. Additionally, the
30 combination of cyclosporin A and cobra venom factor was
31 studied. Adachi et al report that "in our study, there
32 was no statistically significant difference in mean
33 survival time between the group treated with

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1 cyclosporin A and cobra venom factor and the group
2 treated with cobra venom factor alone". Mean survival
3 times were between 40 and 50 hours. Kemp et al
4 (*Transplant. Proc.* XIX (6) 4471-4474 (1987)) also
5 studied various potential discordant xenograft survival
6 promoters in a study of rabbit kidney xenografts into
7 cats. Candidate survival promoters included captopril,
8 enalapril, nifedipine, prostacyclin, sodium
9 chromoglycate, an azathioprine/corticosteroid
10 combination, cyclosporin A and cobra venom factor.
11 Hyperacute rejection was not prevented by any of these
12 candidate drugs except for cobra venom factor which,
13 the authors state, "had a striking effect, with
14 prolongations in graft survival up to seven days".

15
16 Concordant xenograft rejection has been studied by
17 Hardy et al (*Transplantation* 33 (3) 237-242 (1982)),
18 who examined the effect of selective lymphoid
19 irradiation (SLI) with palladium-109-haematoporphyrin
20 combined with antilymphocyte globulin, a therapy which
21 is primarily directed against T-cell-mediated
22 rejection. Mean survival times were in the order of
23 12.5 days, compared with untreated controls which
24 survived for 2.9 days on average. Homan et al
25 (*Transplantation* 31 (3) 164-166 (1981)) studied the
26 effect of cyclosporin A in the promotion of concordant
27 xenograft survival. The best graft survival was for a
28 medium of 21 days, compared to 2 days for untreated
29 controls. Monden et al (*Transplantation* 43 (5) 745-746
30 (1987)) studied hamster-to-rat liver xenografts and
31 found that cyclosporin A had no significant graft
32 survival promoting effect when compared to untreated
33 controls. Valdevere et al (*Transplant. Proc.* XIX (1)



1 1158-1159 (1987)) also studied hamster-to-rat liver
2 xenografts and found a significant improvement in graft
3 survival time, compared to controls, when cyclosporin A
4 was administered in conjunction with splenectomy;
5 survival was improved from an average of 3.6 days to an
6 average of 17.6 days. Knechtle et al (Transplant.
7 Proc. XIX (1) 1137-1139 (1987)), like the Hardy et al
8 group referred to above, studied the effect of total
9 lymphoid irradiation (TLI), but this time in
10 conjunction with cyclosporin A therapy, on the
11 promotion of concordant xenograft survival. They
12 report that, in hamster-to-rat cardiac xenografts,
13 cyclosporin A in high doses significantly prolonged
14 survival, but only an occasional recipient had
15 long-term graft function. TLI in combination with
16 cyclosporin A significantly further promoted graft
17 survival.

18

19 Conventional therapy against allograft rejection is to
20 use cyclosporin A either on its own or in combination
21 with steroids or azathioprin or both. In the opinion
22 of some, the combination of triple therapies does not
23 represent an improvement over therapy using cyclosporin
24 A alone. These therapies are effective in the
25 prevention and reversal of acute graft rejection, and
26 probably retard the onset of chronic graft rejection,
27 but appear to have little or not effect on the reversal
28 of chronic rejection after onset.

29

30 Knechtle et al (Heart Transplantation & Immunology IV
31 No 5: 541-545 (Sept/Oct 1985)) have analysed the effect
32 in rats of cyclosporine, total lymphoid irradiation and
33 cobra venom factor on hyperacute rejection. It was



5a

concluded that the addition of total lymphoid irradiation or cyclosporine to treatment with cobra venom factor did not result in longer survival than cobra venom factor alone.

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The present invention seeks to promote the better survival of, or to rescue from rejection, concordant xenografts and/or allografts. The invention is based on the realisation that concordant xenograft survival,

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1 and also allograft survival, can be markedly enhanced
2 by treatment with a combination of cyclosporin A and
3 cobra venom factor, or by combinations of compounds
4 having similar modes of action.

5

6 According to a first aspect of the present invention,
7 there is provided a pharmaceutical formulation
8 comprising a complement inhibitor and an
9 immunosuppressant with cyclosporin A-like activity.

10

11 Pharmaceutical formulations in accordance with the
12 invention therefore have two components, at least. A
13 first component is a complement inhibitor. The
14 complement inhibitor may either prevent the activation
15 of complement or remove or deplete it, or one or more
16 of its components. Preferably, the complement
17 inhibitor will inhibit both the classical and
18 alternative pathways of complement activation. It is
19 therefore preferred that the complement inhibitor acts
20 to block the pathway at C3, whether by inhibition or
21 removal or depletion. It will be appreciated,
22 therefore, that antibodies (for example monoclonal
23 antibodies) against C3 may be used as the complement
24 inhibitor in the present invention. Other useful
25 complement inhibitors include soluble decay activating
26 factor (DAF) and soluble membrane cofactor protein
27 (MCP). One of the most preferred complement inhibitors
28 is cobra venom factor.

29

30 Snake venoms are among the most complex of animal
31 secretions, containing a vast number of substances with
32 different pharmacological and biochemical activities.
33 The presence in venoms of principles affecting



1 complement has been known for almost a century. The
2 action of the venom of the cobra (*Naja naja kaouthia*)
3 on serum complement has been studied in some detail.
4 The active factor is a glycoprotein with a molecular
5 weight of 144kDa, which combines with serum factor B in
6 the presence of magnesium ions. Factor B is then split
7 into two components by the proteolytic action of serum
8 factor D, creating Bb and Ba. The complex CoF-Bb,
9 where CoF represent cobra venom factor, is a highly
10 active C3- and C5-splitting enzyme or convertase.
11 However, unlike normal C3- and C5-convertases, the
12 CoF-Bb is resistant to the normal complement regulatory
13 systems. Therefore, its action will continue, until
14 all C3 has been converted, thus effectively depleting
15 the individual of complement activity. Splitting of C5
16 releases C5a, which has anaphylactoid action; not all
17 *Naja* cobra venom factors possess this C5 convertase
18 activity, but this is not believed to be important for
19 the purposes of the present invention. Inhibition of
20 complement is not restricted to venoms of the *Naja*
21 genus. Eggertsen et al (*Toxicon* 18 87-96 (1980)) have
22 studied venoms from snakes of the families Elapidae
23 Viperidae and Crotalidae. Of 26 venoms studied, 22
24 have the ability to inhibit the activity of normal
25 human complement. Analysis of the structure of cobra
26 venom factor suggests that it is the equivalent of
27 cobra C3 or a partially degraded product of it.

28
29 Cobra venom factor, and other snake venom factors, will
30 generally not be administered in the present invention
31 in their native form, because of the obvious problems
32 of toxicity. In the absence of other measures to
33 combat the toxicity problem, therefore, it will



1 generally be necessary at least partially to purify the
2 active factor from the crude venom. It is not
3 necessary for cobra or other snake venom factor to be
4 purified to homogeneity for use in the present
5 invention, but clearly regulatory problems if nothing
6 else would tend to prescribe the use of a substantially
7 purified factor. Cobra venom factor, as a
8 semi-purified preparation, can be obtained from
9 chemical suppliers such as Sigma (Sigma Chemicals
10 Limited, Poole, Dorset), who also supply crude cobra
11 venom for in-house purification. An example of a
12 preparative process for cobra venom factor from crude
13 cobra venom is given in the examples.

14
15 It will be appreciated that, in addition to the use of
16 natural cobra venom factor and other snake venom
17 factors, analogues of such factors (including active
18 fragments) can be used in this invention, as can
19 synthetic factors including those produced by
20 recombinant DNA technology. The characteristic of CoF
21 that makes it suitable for use in the present invention
22 is its ability to form a stable complex with factor Bb
23 which is resistant to the normal breakdown mechanism.
24 Other molecules having this characteristic are
25 therefore also preferred for use in the invention.

26
27 A second component of pharmaceutical formulations in
28 accordance with this invention is an immunosuppressant
29 with cyclosporin A-like activity. Such compounds will
30 be well known to those skilled in the art and include
31 cyclosporins other than cyclosporin A, such as
32 cyclosporin C and cyclosporin G. Eicosanoids also have
33 cyclosporin A-like activity and include PGI_2



(prostacyclin), PGE₂, PGD₂, rapamycin and FK 506, (Fujisawa). Compound FK 506 is one of those that is preferred for use in the present invention, but the most preferred is cyclosporin A itself. Cyclosporin A is available from Sandoz under the trademark SANDIMUN.

A particularly preferred embodiment of the present invention uses a pharmaceutical formulation which comprises cobra venom factor and cyclosporin A, two of the most preferred individual compounds discussed above.

It will be appreciated that other ingredients may be present in pharmaceutical formulations for use in accordance with the invention. Excipients will usually be present, and the presence of other active ingredients is not excluded. More than one active ingredient of each category may be present.

It will be appreciated that the active ingredients do not necessarily have to be co-administered in a single formulation. A product containing a complement inhibitor and an immunosuppressant with cyclosporin A-like activity can be used as a combined preparation for simultaneous, separate or sequential use in promoting allograft or concordant xenograft survival or rescue from rejection.

A kit comprising a pharmaceutical preparation of a complement inhibitor and a pharmaceutical preparation

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1 of an immunosuppressant with cyclosporin A-like activity
2 may be used.

3
4 Cyclosporin A and other immunosuppressants with
5 cyclosporin A-like activity will usually be
6 administered parenterally in the practice of the
7 present invention. In such cases, the preparation
8 containing the immunosuppressant will be sterile.
9 However, cyclosporin A itself, and some other
10 immunosuppressants, may be administered orally, in
11 which case sterility is not essential. Cyclosporin A
12 itself is only sparsely soluble in water and for
13 practical purposes it is preferred to dissolve it in an
14 oil, such as a vegetable oil. Olive oil has been found
15 to be acceptable in practice. Dissolution in oil is
16 only necessary when dealing with an immunosuppressant
17 which is not soluble in water, and in general terms,
18 the immunosuppressant may be dissolved in any
19 appropriate physiologically acceptable carrier. In
20 some cases, dissolution may not even be necessary. The
21 concentration at which the immunosuppressant is
22 prepared will of course depend on the nature of the
23 immunosuppressant itself and the intended dose, which
24 will generally be under the guidance of the clinician
25 or physician. As a general guide, however, it has been
26 found appropriate to prepare formulations of the
27 cyclosporin at a concentration of from 1 to 100 mg/ml,
28 for example from 1 to 10 mg/ml, typically about 5 mg/ml
29 for parenteral purposes or 25 to 100 mg/ml, for example
30 from 50 to 100 mg/ml, typically about 100 mg/ml for
31 oral administration.

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1 Complement inhibitors such as cobra venom factor will
2 generally be given parenterally and for this purpose
3 will generally be sterile. Cobra venom factor itself
4 may be formulated in phosphate buffered saline (without
5 azide) or any other suitable solvent or carrier. It
6 may be that oral formulations can be developed. The
7 concentration of complement inhibitor in a
8 pharmaceutical formulation will again depend on the
9 ultimate intended dose, which will be under the control
10 of the clinician or physician. For guidance, however,
11 a preparation of cobra venom factor in PBS can be made
12 at a concentration ranging from 0.05 mg/ml up to the
13 limit of solubility, which will be dependent on its
14 purity. Compositions having a concentration up to
15 5 mg/ml may be useful in practice, for example those
16 having a concentration of from 0.1 to 2 mg/ml, for
17 example about 0.5 mg/ml.

18

19 Because cyclosporin A is essentially not soluble in
20 aqueous media, whereas cobra venom factor is, these two
21 preferred active ingredients will generally be
22 formulated separately and administered separately in
23 practice. This is not in principle essential, however,
24 as it may be possible to formulate an appropriate
25 emulsion of the two active ingredients, and different
26 combinations of active ingredients may be soluble in a
27 common solvent or at least suspendable in a common
28 carrier.

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30 Reference has been made above to parenteral injection.
31 The preferred site of injection is muscle, because of
32 the depot effect resulting from an intramuscular
33 injection. Intravenous injection, however, may be

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1 appropriate in some circumstances and subcutaneous
2 injection may have some if not all of the advantages of
3 intramuscular injection. Intraperitoneal injection
4 could be used if circumstances dictate.

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19 It may be preferable in many circumstances to
20 pre-administer some or all of the active ingredients
21 useful in this invention before graft surgery takes
22 place. It should also be noted that a primary
23 advantage of the present invention as it relates to
24 allografts is that the combination of active
25 ingredients useful in the present invention would be
26 useful for preventing chronic rejection or managing
27 chronic rejection (ie rescuing from rejection) after
28 its onset; this usually happens many years
29 post-transplant.

30

31 Allograft recipients suffering from chronic graft
32 rejection, or at risk from chronic graft rejection, may
33 well be receiving immunosuppression with cyclosporin A



1 or another immunosuppressant having cyclosporin A-like
2 activity. For the purposes of this invention, in order
3 to treat or prevent chronic graft rejection in such
4 patients it is necessary only to add complement
5 inhibitor therapy. According to a fifth aspect of the
6 invention, there is provided the use of a complement
7 inhibitor in the preparation of an agent for promoting
8 allograft or concordant xenograft survival, or rescue
9 of such a graft from rejection, in a graft recipient
10 being treated with an immunosuppressant with
11 cyclosporin A-like activity. The invention therefore
12 has application in a method of promoting allograft or
13 concordant xenograft survival and/or of rescuing such a
14 graft from rejection, in a host, the method comprising
15 administering to a graft recipient or an intended graft
16 recipient, being treated with an immunosuppressant with
17 cyclosporin A-like activity, a complement inhibitor.

18
19 The immunosuppressant with cyclosporin A-like activity
20 will be administered at any suitable effective but non-
21 toxic level, having regard to the species being treated
22 and the organ being transplanted. The upper limit of
23 cyclosporin A tolerance in humans is determined by its
24 nephrotoxicity and the age of the recipient. 20 mg/kg
25 per day may be a safe upper limit for treating humans
26 in many cases, although other species may well have
27 higher tolerances. Cyclosporin A is effective at low
28 doses, and so the lowest amount that may be
29 administered will simply be the lowest amount that
30 gives an effective response. Amounts of 1 or 2 mg/kg
31 per day have in some cases found to be effective. As a
32 general guide, between 5 and 10 mg/kg per day can be
33 administered. If administered parenterally,



1 cyclosporin A may be given on alternate days, in which
2 case the dose would be doubled. For the preferred,
3 oral route, however, daily dosage will be the regimen
4 of choice; more particularly cyclosporin A may be
5 administered twice or more frequently per day to give a
6 total daily dose in the order of 5 to 10 mg/kg, for
7 preference. Other immunosuppressants with cyclosporin
8 A-like activity will be administered at effective but
9 non-toxic doses, as will be determined by the clinician
10 or physician.

11

12 The complement inhibitor, such as cobra venom factor,
13 will as a guiding principle be administered in an
14 amount which effectively inhibits complement activity,
15 as determined by the complement haemolysis₅₀ (CH₅₀)
16 assay. The safe upper limit for the administration of
17 cobra venom factor has not been determined, but doses
18 of 2 mg/kg do not appear to be toxic. The minimum
19 dosage of cobra venom factor is species dependent and
20 can readily be determined by the CH₅₀ assay. Daily
21 doses of between 0.01 and 0.1 or 0.2 mg/kg have been
22 found to be effective for cobra venom factor.
23 Effective and non-toxic dosages of other complement
24 inhibitors can also readily be determined by those
25 skilled in the art, for example, using the CH₅₀ assay.

26

27 Cobra venom factor takes about 6 hours for its initial
28 activity to take effect after intramuscular
29 administration. Once the initial activity is observed,
30 it can last for more than 1 day, for example 2 days,
31 and so daily administration of cobra venom factor may
32 not be necessary. In fact, it is preferred to
33 administer cobra venom factor on alternate days, in



1 which case the preferred daily dosages given above will
2 be doubled on the day of administration. In practice,
3 it has been found that a particularly suitable dosage
4 regimen is to administer 0.4 mg/kg intramuscularly on
5 alternate days.

6
7 Cyclosporin A or other immunosuppressant administration
8 will usually be continuous. Cobra venom factor or
9 other complement inhibitor administration will
10 generally not be continuous, but may be prolonged; even
11 this may not in practice be essential, as short term
12 therapy may be all that is needed for the treatment
13 for, or rescue from rejection of, concordant
14 xenografts.

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20 The invention will now be illustrated by reference to
21 the following preparation and examples. The examples
22 refer to the accompanying drawings, in which:

23

24 FIGURE 1 is a graph of hamster heart xenograft survival
25 in rats, with percentage survival being plotted against
26 days post transplant; and

27

28 FIGURE 2 is a graph of hamster heart xenograft survival
29 in cyclophosphamide treated rats; the graph otherwise
30 corresponds to Figure 1.

31

32

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1 Preparation

2

3 This preparation gives a protocol for preparing cobra
4 venom factor from crude cobra venom.

5

6 1g of venom of *Naja naja kaouthia* (Sigma, Product No.
7 V-9125, Lots Nos. 116F-0398 and 58F-0565) was dissolved
8 in 60 ml of degassed sodium phosphate buffer (0.01M) at
9 pH 7.5 and pumped onto a 90cm x 26mm (internal
10 diameter) column of DEAE cellulose (DE52, Whatman) and
11 equilibrated with the same buffer. The column was then
12 eluted at 43.0 ml per hour with the starting buffer
13 until the A_{280} absorbence of the effluent returned to
14 0. A linear 3 litre:3 litre gradient of starting
15 buffer and limit buffer (0.01M sodium phosphate, pH
16 7.5, containing 0.5M sodium chloride) was then pumped
17 on at 40.0 ml per hour and 7.0 ml fractions were
18 collected. Fractions were tested for anti-
19 complementarity, and the positive fractions were pooled
20 and concentrated on CX-10 ultrafilters.

21

22 The whole of the cobra venom factor pool was pumped
23 onto a 90cm x 26mm (internal diameter) column of
24 Sephadex G200 (fine) (Pharmacia GB Limited, London),
25 equilibrated with PBS (no azide) and eluted with PBS at
26 36 ml per hour. 6 ml fractions were collected and
27 assayed for anti-complementarity activity. The cobra
28 venom factor fractions (ie the active fractions) were
29 pooled, especially on the descending limb of the peak,
30 concentrated on CX-10 ultrafilters, partitioned into
31 aliquots and stored frozen at -20°C ready for use.

32

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1 The anti-complementary activity of cobra venom factor
2 referred to above can be measured by the following
3 method. 20 μ l fractions were incubated with 20 μ l of
4 fresh human serum for 20 minutes at 37°C. 5 μ l amounts
5 were then added to wells in a test plate, made from:

6

7 5.0 ml 2% aqueous agarose;
8 4.5 ml double strength PBS including 20mM EDTA;
9 0.5 ml 10% washed guinea pig erythrocytes; and
10 0.5 ml fresh normal human serum.

11

12 The plate was then incubated overnight at +4°C.
13 Positives are shown by clear zones of lysis after
14 incubation.

15

16 Example 1 (Comparison Example)

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18 In this example, the rats are DA rats from Banting and
19 Kingdom, and the hamsters are Syrian hamsters, also
20 from Banting and Kingdom. Following the method of
21 Heron (*Acta Pathol. Microbiol. Scand.* 79 366-372
22 (1971)), hamster hearts were grafted into the necks of
23 the rats. The aorta was joined to the carotid artery
24 using the cuff technique described by Heron.
25 Similarly, the pulmonary artery was joined to the
26 jugular vein. All other cardiac vessels were ligated.
27 Hearts began beating with a few minutes of removal of
28 clamps. Skin was then closed over the implanted heart,
29 which was monitored daily by external palpation.

30

31 Heart graft survival data are shown in Figure 1 and are
32 represented by line 1. It can be seen that the median
33 survival rate of the grafts was 3 days.



1 Example 2 (Comparison Example)

2

3 The procedure of Example 1 was repeated, except that
4 cyclosporin A was administered at a dosage of 20 mg/kg
5 per alternate day. The cyclosporin A was administered
6 as a 20 mg/ml preparation in olive oil and injected
7 intramuscularly, with the first dose being given at the
8 time of transplantation. Injections were given on
9 alternate days thereafter. Graft survival data are
10 again shown in Figure 1: line 2 gives the results from
11 this example. The median graft survival rate is 3
12 days.

13

14 Example 3 (Comparison Example)

15

16 The procedure of Example 1 was repeated, except that
17 cobra venom factor was administered at a dosage of 0.4
18 mg/kg per alternate day. The cobra venom factor was
19 administered as a 0.5 mg/ml solution in azide-free PBS.
20 The first injection was made intramuscularly at the
21 time of transplantation, and subsequent intramuscular
22 injections were made on alternate days thereafter. The
23 graft rejection data are again presented in Figure 1.
24 The results are shown on line 3, which indicates that
25 the median survival rate for the grafts is 6 days.

26

27 Example 4

28

29 The procedure of Example 1 was repeated, except that
30 both cyclosporin A and cobra venom factor were
31 administered to the recipient. Cyclosporin A was
32 administered at a dose of 20 mg/kg on alternate days,
33 so that the conditions for cyclosporin administration



1 were the same as in Example 2. The cobra venom factor
2 was administered under the same conditions as described
3 in Example 3. Both the cyclosporin A and the cobra
4 venom factor were first administered at the time of
5 transplantation. Graft survival data are again shown
6 in the figure. In this example, no graft rejection is
7 observed, as can be seen from line 4 of Figure 1. At
8 present, median graft survival is in excess of 50 days.

9
10 Example 5

11
12 The procedure of Example 4 was repeated, except that
13 cobra venom factor administration was discontinued
14 after 28 days post transplant. Median graft survival
15 was greater than 100 days. Two animals were sacrificed
16 at 103 and 105 days and histological examination of the
17 graft showed normal cardiac architecture. A remaining
18 rat has survived with a beating hamster heart for more
19 than 5 months so far.

20
21 Example 6 (Comparison Example)

22
23 20 rats were given hamster hearts by the protocol
24 described in Example 1. They were treated with
25 cyclosporin A as described in Example 2, and in
26 addition they were given ENDOXAN cyclophosphamide i.p.
27 (20 mg/kg) one day pre-transplant (day-1) and on day 2
28 post-transplant. (The word ENDOXAN is a trade mark for
29 cyclophosphamide, which is an inhibitor of antibody
30 production.) The hamster hearts were rejected as shown
31 in line 5 of Figure 2. The observed delayed rejection,
32 in which the delay was a result of the short
33 cyclophosphamide therapy, simulates a delayed xenograft



1 rejection of the type that would result from inadequate
2 immunosuppressive protocols.

3

4 Example 7

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6 Five rats were treated as in Example 6, but
7 additionally were treated with cobra venom factor as in
8 Example 4 from day 7. Treatment with cobra venom
9 factor lasted for 14 days. All animals were rescued
10 from rejection, as can be seen from line 6 of Figure 2.

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CLAIMS

1. The use of a complement inhibitor and an immunosuppressant with cyclosporin A-like activity in the preparation of an agent or agents for promoting allograft or concordant xenograft survival or rescue from rejection; wherein said allograft survival or rescue from rejection is in respect of an allograft which is not hyperacutely rejected.
2. The use of a complement inhibitor in the preparation of an agent or agents for promoting allograft or concordant xenograft survival, or rescue from rejection, in a graft recipient being treated with an immunosuppressant with cyclosporin A-like activity, wherein said allograft survival or rescue from rejection is in respect of an allograft which is not hyperacutely rejected.
3. The use as claimed in claim 1 or claim 2, wherein the complement inhibitor inhibits both the classical and alternative pathways of complement activation.
4. The use as claimed in any preceding claim, wherein the complement inhibitor comprises MCP, DAF or an antibody against C3.
5. The use as claimed in claim 1 or claim 2, wherein the complement inhibitor comprises a molecule which forms a stable complex with factor Bb, wherein the complex is resistant to normal breakdown mechanisms.
6. The use as claimed in any of claims 1 to 3, wherein the complement inhibitor comprises cobra venom factor.



7. The use as claimed in any preceding claim, wherein the immunosuppressant with cyclosporin A-like activity is a cyclosporin or an eicosanoid.

5 8. The use as claimed in claim 7, wherein the immunosuppressant is cyclosporin A.

9. The use as claimed in claim 7, wherein the immunosuppressant is FK 506.

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10. The use according to any preceding claim, wherein the complement inhibitor and the immunosuppressant with cyclosporin A-like activity are for simultaneous, separate or sequential administration.

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11. The use according to any preceding claim, wherein the complement inhibitor and the immunosuppressant with cyclosporin A-like activity are provided in kit form.



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CLAIMS FOR THE CONTRACTING STATES: ES and GR

1. A process for the preparation of a pharmaceutical formulation comprising admixing a complement inhibitor and an immunosuppressant with cyclosporin A-like activity.
2. A process as claimed in claim 1, wherein the complement inhibitor inhibits both the classical and alternative pathways of complement activation.
3. A process as claimed in claim 1 or 2, wherein the complement inhibitor comprises MCP, DAF or an antibody against C3.
4. A process as claimed in claim 1, wherein the complement inhibitor comprises a molecule which forms a stable complex with factor Bb, wherein the complex is resistant to normal breakdown mechanisms.
5. A process as claimed in claim 1 or 2, wherein the complement inhibitor comprises cobra venom factor.
6. A process as claimed in any one of claims 1 to 5, wherein the immunosuppressant with cyclosporin A-like activity is a cyclosporin or an eicosanoid.
7. A process as claimed in claim 6, wherein the immunosuppressant is cyclosporin A.
8. A process as claimed in claim 6, wherein the immunosuppressant is FK 506.



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- 5 9. A process for the preparation of a product containing a complement inhibitor and an immunosuppressant with cyclosporin A-like activity for use as a combined preparation for simultaneous, separate or sequential use in promoting allograft or concordant xenograft survival or rescue from rejection.
- 10 10. A process for the preparation of a kit comprising assembling a pharmaceutical preparation of a complement inhibitor and a pharmaceutical preparation of an immunosuppressant with cyclosporin A-like activity.
- 15 11. The use of a complement inhibitor and an immunosuppressant with cyclosporin A-like activity in the preparation of an agent for promoting allograft or concordant xenograft survival or rescue from rejection.
- 20 12. The use of a complement inhibitor in the preparation of an agent for promoting allograft or concordant xenograft survival, or rescue from rejection, in a graft recipient being treated with an immunosuppressant with cyclosporin A-like activity.



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