EARLY EVALUATION OF Ia MONOCLONAL ANTIBODIES IN PROLONGING NON-HUMAN PRIMATE SKIN ALLOGRAFT SURVIVAL

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Summary

Monoclonal antibodies (MCA) specific against one subset of lymphocytes (anti-B) were used in treating skin allograft rejection in Rhesus monkeys. Rhesus monkeys were treated daily with intravenous MCA until the skin graft was rejected. The dose of the MCA was adjusted according to the peripheral B-cell counts obtained by rosetting technique as well as by indirect immunofluorescence. We finally achieved a safe dose, though the margin of safety was narrow. We also noted that the B-cell count dropped to a significant degree and that the survival of the skin graft was prolonged.

Introduction

Basic immunological research has recorded a number of dramatic advances such as the recent demonstration by Kohler and Milstein [1] that by fusing specifically immune B-cells from mice to murine myeloma cells, hybrid cells can be produced that will grow in continuous cell culture, thereby providing an infinite source of homogenous antibody with specificity for a single antigen. A number of hybridomas secreting monoclonal antibodies to various human cell surface antigens have been described [2]. The existence of various forms of lymphocytes and even subsets within each form are already known. In clinical organ transplantation, up to the present time, the immunosuppressive drugs are directed against the bulk of all kinds of cells in spite of the differences in their actions. It would be more appropriate if there were available immunosuppressive reagents that were more specific for certain specialised cells of the immune system. With the advent of monoclonal antibodies against various leucocyte subpopulations, this possibility is now within the realm of realisation. These reagents are not only specific, but are also very potent and could be used to eliminate a particular subpopulation of lymphocytes that would normally take part in allograft rejection. To investigate this possibility we have treated Rhesus monkeys with monoclonal antibodies
against human Ia antigens (which also cross react with monkeys’ Ia) for three main purposes: (a) to determine if they are safe and non-toxic; (b) to ascertain the dose by monitoring the peripheral B-cell counts; and (c) to decide if the skin allograft can be prolonged by their use.

Materials and methods

Animals

Young male or female Rhesus monkeys from the California Primate Research Center were used for this experiment. Initially (first two), monkeys suffering from endometriosis were used as we were expecting fatality in adjusting the initial dose. Later healthy animals weighing between 5 and 6kg were used for this experiment.

Monoclonal antibodies

Female BALB/C mice (6 weeks old) were immunised intravenously with $2 \times 10^7$ Ia positive leukaemia cells (Reh) at 14 day intervals. Four days after the third immunisation the spleens were removed and a suspension of single cells was prepared. Cell fusions were carried out by fusing with mouse myeloma cells (X63) according to the procedure developed by Kohler and Milstein [1]. After cell fusion the cells were distributed and cultured in selective medium. One hybrid clone produced a cytotoxic monoclonal antibody (H4) that reacted with B-cells and other Ia positive cells but not T-cells. It was an IgG3 antibody that bound to protein A. By immunoprecipitation technique and gel electrophoresis, H4 reacted with the human Ia-like antigen (P2735). The H4 ascites had a titre against monkey B-cells to $1:10^5$. It was filter sterilised and checked for pyrogens. The dose of H4 given to an animal varied according to the titre. Twenty to 500 lambda diluted with 6ml of normal saline were usually given intravenously.

Monitoring

The total and differential counts of peripheral white blood cells were done daily. The peripheral B-cells were monitored daily by a rosetting technique using ox red blood cells coupled with the mouse monoclonal antibody. Rosetted cells were counted on a haemocytometer and expressed as a percentage of total lymphocytes. Additionally, B-cells were tested for indirect immunofluorescence using the monoclonal antibody. Using these techniques, it was possible to define the dosage and the timing of antibody administration required to provide in vivo coating of the reactive Ia positive cells in Rhesus monkeys.

Procedure

Monoclonal antibodies (MCA) were given intravenously to arrive at a safe dose initially. Thereafter, the dose was increased or decreased according to the peripheral B-cell count. We administered MCA two days prior to skin grafting. A full-
thickness skin graft 4cm x 4cm was applied on the forearm. The forearm was put in a plaster cast which was removed on the fifth postoperative day. Discolouration of the graft was taken as the end-point for graft rejection.

Results

Efficacy

Results of all the experiments are summarised in Table I. Ten animals were used in this experiment. Initially three died while we were still trying to ascertain the correct dose. Thereafter there was only one death that occurred on the sixth postoperative day, due to a suddenly increased MCA dose. This left six animals alive and well. One of them was a technical failure. The male primate with large incisors chewed the cast and the graft. All five grafts that remained had prolonged survival. The monoclonal antibody used was effective in prolonging skin graft survival two to three times that of the control.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Animal No.</th>
<th>Titre</th>
<th>Dose/ Lambda</th>
<th>Skin grafted or not</th>
<th>Survival of skin graft</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7895</td>
<td>$10^5$</td>
<td>1000</td>
<td>no</td>
<td>NA</td>
<td>died</td>
</tr>
<tr>
<td>2</td>
<td>8048</td>
<td>$10^5$</td>
<td>500</td>
<td>no</td>
<td>NA</td>
<td>died</td>
</tr>
<tr>
<td>3</td>
<td>7898</td>
<td>$10^4$</td>
<td>250</td>
<td>no</td>
<td>NA</td>
<td>died</td>
</tr>
<tr>
<td>4</td>
<td>7565</td>
<td>$10^5$</td>
<td>20–50</td>
<td>yes</td>
<td>19 days</td>
<td>alive and well</td>
</tr>
<tr>
<td>5</td>
<td>7681</td>
<td>$10^4$</td>
<td>100–500</td>
<td>yes</td>
<td>–</td>
<td>animal chewed the graft through the cast in the upper arm on the 5th day alive and well</td>
</tr>
<tr>
<td>6</td>
<td>7531</td>
<td>$10^4$</td>
<td>500</td>
<td>yes</td>
<td>14 days</td>
<td>alive and well</td>
</tr>
<tr>
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<td>7862</td>
<td>$10^4$</td>
<td>50–75</td>
<td>yes</td>
<td>16 days</td>
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<tr>
<td>8</td>
<td>7484</td>
<td>$10^4$</td>
<td>100–250</td>
<td>yes</td>
<td>13 days</td>
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<tr>
<td>9</td>
<td>6596</td>
<td>$10^3$</td>
<td>50–125</td>
<td>yes</td>
<td>15 days</td>
<td>alive and well</td>
</tr>
<tr>
<td>10</td>
<td>6587</td>
<td>$10^3$</td>
<td>100–500</td>
<td>yes</td>
<td>–</td>
<td>Died on 6th day with viable graft</td>
</tr>
</tbody>
</table>

Safety

As no prior information exists in the literature, we started with 1.0ml, 0.5ml and 0.25ml, respectively, in the first three animals. All these doses proved to be fatal and the animals died within hours of receiving the antibodies. We felt that the cause of death was directly related to the cytotoxic effect of the H4 ascites fluid, because a similar volume of ascitic fluid with a lower cytotoxic titre did not have any untoward effects on the primates. In all other animals there were no adverse reactions except tachycardia. Seven Rhesus monkeys which remained alive and well were treated for two days with the ascitic form of the monoclonal reagent.
H4 before giving the full-thickness skin graft on the third day. Their peripheral B-cells were monitored daily by a rosetting technique as well as by indirect immunofluorescence. Discolouration of the graft was considered as rejection even if they were neither shrivelled nor lost in part or whole. We felt an earlier but standard end-point would be useful in saving more H4 reagents than continuing for three or four additional days.

Discussion

In organ transplantation up to the present time, the immunosuppressive drugs have been directed against the bulk of the immune system in spite of the difference in the action of its components. The possibility of having an immunosuppressive agent that will act specifically against only one subset of cells is an exciting one. This possibility has now been realised with the advent of monoclonal antibodies against leucocyte subpopulations. These reagents are not only specific but also are very powerful and can be used to eliminate particular subpopulations of lymphocytes that are involved in tissue rejection. The monoclonal OKT3 has been used to lower circulating T cells in patients who are showing acute rejection of an allografted kidney.

To achieve the safe level of dosage, we started at a relatively high dose and lost three of our animals, the cause of death being anaphylactic reaction. Maybe the mast cells (which also possess B-cell surface antigens) were also destroyed, releasing histamine, which in turn was responsible for acute pulmonary oedema and death. Total body B-cell depletion as a cause of death is difficult to accept, as time is needed for overwhelming sepsis to set in. Our animals died between one and twelve hours after receiving the MCA. We now know that for a monoclonal antibody as potent as ours, a much smaller dose is needed. The lethal effects of antibody H4 appears to be related to its Ia specificity because 2ml of another monoclonal ascites specific for human leiomyosarcoma to a titre of 1:64,000 had no adverse effects on similar monkeys [4].

As far as the prolongation of the skin grafts are concerned, we know that we are increasing the graft survival in an indirect way through B-cell depletion, instead of depleting the T cytotoxic or T helper cells. However, the very fact that we obtained 2–3 times prolongation compared to the control animals is a proof that this MCA is specific and effective. The absolute count of B-cells in the peripheral blood dropped. It took two to three days to achieve this objective and MCA had to be administered continually lest the B-cells ‘rebound’. Our MCA did not have any anti-platelet effect.

Unfortunately, at the present time all hybridomas rarely result in the production of antibodies derived from the second species; thus, the production of human hybridoma antibodies will require the isolation and characterisation of a human myeloma cell which can be drug-marked and grown in continuous tissue culture [5]. Such human myeloma cells could then be fused with human peripheral B-cells for the production of reagents that could be used therapeutically. The list of potential applications of this reagent is endless, limited only by the imagination of the investigators [5]. Doubts can also be raised about injecting secreted products of malignant human cells into patients.
On the basis of our study, we feel that anti-human Ia antiserum is perhaps a bit dangerous to use in clinical studies at this time. But our study does raise the exciting possibility that other monoclonal reagents which react with antigens less ubiquitous than Ia and have a greater margin of safety, may eventually be used to replace today’s harmful pan-cytotoxic drugs.

Acknowledgment

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References

1  Kohler G, Milstein C. *Nature* 1975; 256: 495
3  Cosimi AB et al. *Abstract Book American Society of Transplant 1981*: 1
5  Kazmar RE, Fathman CG. *Mayo Clinic Proc* 1980; 55: 517

Open Discussion

TRAEGGER (Chairman)  Do you think it would be better to use very specific monoclonal antibodies acting on a special T or B cell or do you think it would be better to get a monoclonal antibody with a broad spectrum against T cells?

CHATTERJEE  That is a very good question, but this is, as you see, a pioneer study. As you know our colleagues from Boston have already presented in the American Society of Transplant Surgeons meeting, in very few patients, a small amount of data but with an impressive result, using monoclonal antibody against cytotoxic T cells. Very soon you may have a combination of anticytotoxic, anti-B and antimacrophage, so you will be leaving all these suppressor cells behind and perhaps you will never have a graft rejection. It is just a dream but at least the possibility is there and we should try to achieve it.