# The effect of abrin, anti-lymphocyte globulin and their conjugates on the immune response of mice to sheep red blood cells

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*Summary.* A chemical conjugate of anti-mouse lymphocyte globulin and abrin, is about twice as effective as a similar conjugate made with normal immunoglobulin and abrin, in suppressing the ability of the mouse to respond immunologically to an injection of sheep red blood cells.

Moolten et al.[2-4] have shown diphtheria toxin, covalently attached to antibody, to be more effective in destroying target cells in vitro than a non-antibody conjugate and also to be capable of producing effects in the living animal. Our own previous demonstrations[5,6] that diphtheria toxin in covalent linkage to an antilymphocyte globulin was many times more effective than toxin alone in inhibiting protein synthesis in lymphoblastoid cell lines served to re-emphasise that this mode of attack on mammalian somatic cells was feasible. In the present study we have chosen to use as a model the immune response of the mouse to sheep red blood cells and we have attempted to improve the ability of an anti-mouse lymphocyte globulin (AMLG) to suppress the response by conjugating it with the toxic plant protein, abrin[7].

*Methods.* Abrin was extracted from seeds of *Abrus precatorius* and was purified by affinity chromatography on a column of Sepharose 4B. Conjugates with horse anti-mouse lymphocyte globulin (AMLG) and normal horse immunoglobulin (NIgG) were prepared, isolated and characterised as before[5,6].

To test for immunosuppression, groups of 5, 12-week-old CBA mice were injected i.p. with conjugate and 2 days later with $5 \times 10^8$ sheep red blood cells i.p. After a further 5 days, mice were killed by cervical dislocation, the spleens removed and spleen cells dispersed by gentle sieving. Total white cell counts were made on a Coulter counter Model B and the number of direct plaque forming cells (PFC) estimated[8]. The numbers of PFC per $10^6$ spleen cells were converted to $\log_{10}$ and means and standard deviations computed.

*Results.* As shown in table 1, injection of either abrin or AMLG led to a significant reduction in the numbers of PFC. AMLG was capable of engendering higher levels of immunosuppression than abrin but at very much greater doses. The toxin whilst giving clear immunosuppression at low doses was limited in its application by whole body toxicity. (When given by the i.p. route to groups of 5, 12-

Table 1. Effect of horse anti-mouse lymphocyte globulin and abrin on the immune response of mice to sheep red blood cells. Doses are shown per 25 g b.wt

| Horse anti-mouse lymphocyte globulin | | | | Abrin | | | |
|---|---|---|---|---|---|---|---|
| Dose (moles $\times 10^{-8}$) | Geometric mean PFC | $\bar{x} \div$ SD | p* | Dose (moles $\times 10^{-13}$) | Geometric mean PFC | $\bar{x} \div$ SD | p* |
| 0 | 1092 | 1.11 | | 0 | 1240 | 1.07 | |
| 0.8 | 589 | 1.88 | NS | 0.9 | 880 | 1.43 | NS |
| 1.6 | 171 | 2.75 | 0.01-0.001 | 1.9 | 660 | 1.40 | 0.01-0.001 |
| 3.3 | 159 | 3.08 | 0.01-0.001 | 3.8 | 575 | 1.64 | 0.01-0.001 |

Results are given per $10^6$ spleen cells. * by Student's t-test.

Table 2. Effect of conjugates of horse anti-mouse lymphocyte globulin and normal horse immunoglobulin with abrin on the immune response of mice to sheep red blood cells. Doses are shown per 25 g b.wt

| | Preparation 1 | | Preparation 2 Test 1 | | Test 2 | |
|---|---|---|---|---|---|---|
| Dose (moles $\times 10^{-13}$) | Geometric mean PFC | $\bar{x} \div$ SD | Geometric mean PFC | $\bar{x} \div$ SD | Geometric mean PFC | $\bar{x} \div$ SD |
| AMLG-abrin | | | | | | |
| 0 | 1467 | 1.16 | 1467 | 1.16 | 1837 | 1.15 |
| 3.8 | 637 | 1.44 | 737 | 1.28 | 1148 | 1.20 |
| 7.5 | 473 | 1.37 | 570 | 1.27 | 573 | 1.63 |
| 15.0 | 356 | 1.16 | 324 | 1.29 | 322 | 1.23 |
| 0 no SRBC | 63 | 1.61 | 63 | 1.61 | 174 | 1.25 |
| NIgG-abrin | | | | | | |
| 0 | 1761 | 1.39 | 2141 | 1.06 | 1565 | 1.14 |
| 7.5 | 952 | 1.38 | 1243 | 1.59 | 1108 | 1.45 |
| 15.0 | 769 | 1.20 | 746 | 1.46 | 929 | 1.45 |
| 30.0 | 517 | 1.60 | 634 | 1.70 | 812 | 1.35 |
| 0 no SRBC | 154 | 1.19 | 199 | 1.16 | 146 | 1.24 |

Results are given per $10^6$ spleen cells.

week-old CBA female mice the $LD_{50}$[9] of abrin was $5\times10^{-13}$ moles per 25 g b.wt.) Thus although at the lower levels of immunosuppression abrin was around $10^5$ times more effective than AMLG on a weight for weight basis, the antibody by virtue of its relative lack of whole body toxicity could be used to produce much higher net reductions in the immune response.

Next, tests were carried out to determine the effectiveness of conjugates of abrin with antibody or non-antibody containing immunoglobulin. Each conjugate was made twice, giving AMLG-abrin preparations 1 and 2 and NIgG-abrin preparations 1 and 2. Using the test described earlier it was found that the $LD_{50}$ of the AMLG-abrin was around $43\times10^{-13}$ moles per 25 g b.wt while that of the NIgG-abrin was not significantly different at around $55\times10^{-13}$ moles; conjugation it seemed had reduced the whole body toxicity of abrin about 10-fold. As can be seen in table 2, preparations 1 and 2 were tested once and twice respectively for effects on PFC production. With doses of conjugate in the $7.5-30\times10^{-13}$ moles per 25 g b.wt range, dose related reductions in PFC were obtained and in every instance the decrease was statistically significantly different from the control (dose 0) group. It was a consistent finding that AMLG-abrin was a more potent immunosuppressive agent than was NIgG-abrin, the differential being around 2-fold.

Anti-lymphocyte globulin has been shown to produce effects on phenomena controlled by lymphocytes while leaving other blood elements relatively unaffected[10]. In as much as this may demonstrate its tissue specificity we have chosen anti-lymphocyte globulin as a model for our studies. The initial aim was to increase its effectiveness by attachment of a potent cytotoxic agent. In a sense this has been achieved, the improvement of AMLG-abrin over AMLG alone being around 10,000-fold. However, it appears from the results that the dominant molecular species in the conjugates is abrin, whose ability to immunosuppress is probably not surprising in view of its known cytotoxicity[11] and tendency to localize in spleen[12]. The whole body toxicity of abrin prevented the use of doses sufficient to give very large effects on the response to sheep erythrocytes and precluded the use of mixtures of antibody and toxin in these experiments. It was a major consequence of conjugation to immunoglobulin that there was a reduction of some 10-fold in $LD_{50}$.

The AMLG-abrin appears to be about twice as potent as NIgG-abrin and we are encouraged to think that this is due to the ability of the antibody to localize on to target cells better than does normal immunoglobulin. This, together with the great reduction in toxicity of abrin relative to its effectiveness and the extremely small dose levels employed are seen as promising for this method of developing more specific therapeutic agents.

1 Acknowledgment. We thank Dr Stuart, Society of Herbalists, London, and Prof. G.C. Clark, Dept. of Botany, University of Ghana, for gifts of *Abrus precatorius* seeds, and Mrs L. Bushby for help in the preparation of the horse anti-mouse lymphocyte globulin. D.C.E. is an external member of the staff of the Wellcome Foundation Ltd.

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## Effect of inhibitors of protein synthesis from plants on tobacco mosaic virus infection

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*Summary.* Ricin, abrin, modeccin, gelonin and *Momordica charantia* inhibitor, as well as crude extracts of *Bryonia dioica* seeds and of *Dianthus caryophyllus* leaves, each inhibit protein synthesis in a rabbit reticulocyte lysate and reduce local lesion production by tobacco mosaic virus, thus resembling the effects of the pokeweed antiviral peptide.

Extracts from several plants[2] or seeds[3,4] inhibit replication of plant viruses, and there is evidence that at least some of the active principles may be proteins[2,4,5]. The best characterized is the pokeweed antiviral peptide (PAP) purified from the leaves of *Phytolacca americana*[6,7]. The antiviral activity of this protein is attributed to its inhibitory effect on protein synthesis[8], which in turn is due to enzymic inactivation of the 60S ribosomal subunit[6].

Many seed extracts inhibit protein synthesis[9,10]: from 2 of these, inhibitory proteins have been purified which act in an apparently identical manner to PAP; these are *Momordica charantia* inhibitor[11] (MCI) and gelonin[12], from *Gelonium multiflorum*. These inhibitors, as well as PAP, resemble the A subunit of 3 toxins ricin, abrin and modeccin from the seeds of *Ricinus communis*[13], from seeds of *Abrus precatorius*[13] and from the roots of *Adenia digitata*[14,15] respectively. Each of these toxins consists of 2 polypeptide chains joined by disulphide bonds. The A chain enters cells and inhibits protein synthesis whereas the B chain binds to receptor sites on cell surfaces and behaves like a lectin.

We report now that gelonin, MCI and the toxins mentioned above, like PAP, reduce local lesion production by tobacco mosaic virus (TMV). A crude extract from *Bryonia dioica* seeds, which inhibits protein synthesis, reduces lesion production by TMV, whereas an extract from *Dianthus caryophyllus* leaves, which has antiviral properties, also inhibits protein synthesis.

*Materials and methods.* Ricin[16,17], abrin[19] (abrin C), modeccin[15], MCI[11] and gelonin[12] were prepared as described in the respective references. PAP was a generous gift from Dr