THE ROLE OF METHYLprednisolONE IN THE MODULATION
OF CELLULAR CYTOTOXICITY AND ITS RELATION TO RENAL
TRANSPLANTATION

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Summary

Inhibition of killer cell generation or suppression of their cytolytic capacity once
formed represent fundamental mechanisms by which methylprednisolone may
modulate cellular cytotoxicity in the transplant recipient. In vitro studies in
mixed lymphocyte culture demonstrated that the presence of therapeutic concen-
trations of this agent (0.001-1 µg/ml) during the sensitisation phase resulted
in suppression of lymphocyte activation without cytolysis and marked or total
inhibition of killer cell generation. A considerable individual variation in sensi-
tivity to methylprednisolone-induced suppression was observed and killer cells
once generated were resistant to this agent.

Introduction

In vivo sensitisation by donor histocompatibility antigens following organ
transplantation results in the proliferation and differentiation of recipient T
lymphocyte subpopulations, leading to the generation of specific cytotoxic
killer T cells which mediate acute allograft rejection [1]. Methylprednisolone
(MP) is the major therapeutic agent used to achieve non-specific modulation
of this response in an attempt to facilitate allograft tolerance. Despite con-
siderable study, however, the mechanisms of action of this agent remain un-
clear, and consequently its therapeutic administration is variable and empirical.

In an attempt to elucidate further the effect of this agent at a cellular level,
and to determine whether individual variability in response exists, we have
observed the effects of MP on both cellular proliferation and killer cell genera-
tion in mixed lymphocyte culture (MLC), and on the cytotoxic capacity of
these cells, once formed, as measured in the lymphocyte-mediated cytotoxicity
(LMC) assay.
Materials and Methods

Methylprednisolone was purchased from Upjohn Ltd, and diluted in RPMI 1640 prior to use at final concentrations of 0 – 10 μg/ml in MLC and 0 – 200 μg/ml in LMC assays, the concentrations being selected with reference to those therapeutically obtained in vivo [2].

Cell Preparation

Peripheral blood lymphocytes were obtained from normal control subjects by equilibrium-density centrifugation over Ficoll-Hypaque [3]. Stimulator cells to be used in MLC were irradiated with 61 cobalt, and target cells for LMC assays were PHA-transformed for 72 hours then labelled with 250 μC 51Cr prior to use [4].

Mixed Lymphocyte Culture

100 x 10^3 responder cells and 100 x 10^3 irradiated stimulator cells were cultured in 200 μl of supplemented culture medium (RPMI – 1640 + 2ME + L glut + PS + 10% normal serum) in Linbro round-bottomed microtitre trays in the presence of MP, 0 – 10 μg/ml. Incubation was for 5 days at 37°C in 5% CO₂/95% air. The numerical response was assessed by microscopy, and the percentage proliferation at each concentration calculated from the formula:

\[
\text{percent proliferation} = \frac{\text{cell no (ExP + MP)} - \text{cell no (cont + MP)}}{\text{cell no (Exp - MP)} - \text{cell no (cont - MP)}} \times 100
\]

The incorporation of 3H thymidine was performed as previously described [5], and expressed for each concentration either as cpm or the stimulation index.

The generation of cytotoxic lymphocytes was performed by two methods, in the presence of MP at concentrations of 0 – 10 μg/ml:

(i) micro culture: 3 x 10^5 responder cells and 1 x 10^5 irradiated stimulator cells were cultured for 5 days in 250 μl wells in Falcon flat-bottomed microtitre trays, target cells being added after this time for the assessment of cytotoxicity.

(ii) macro culture: 4 x 10^6 responder cells and 4 x 10^6 irradiated stimulator cells were cultured in supplemented culture medium in 25 ml Falcon flasks. On the 5th day the cells were recovered, resuspended in fresh medium, counted and distributed into microtitre trays for the LMC assays.

Lymphocyte-mediated Cytotoxicity Assays

These were performed using 3 x 10^3 effector cells and 3 x 10^3 51Cr labelled target cells, (100:1 effector:target cell ratio), or by the addition of 3 x 10^3
target cells directly to the microculture wells. Incubation was for 4 and 16 hours at 37°C, and $^{51}$Cr release into the supernatant was measured in cpm using a Searle analytic gamma counter. Total release was achieved by detergent lysis, and percentage specific $^{51}$Cr release was calculated from the formula:

$$\text{Percent specific }^{51}\text{Cr release} = \frac{\text{Exp (cpm) } - \text{Spont (cpm)}}{\text{Total (cpm) } - \text{Spont (cpm)}} \times 100$$

A specific $^{51}$Cr release of greater than 5% and significant by t-test was considered to be positive.

**Results**

*Effect of Methylprednisolone on Cell Numbers in MLC*

Kinetic studies of the proliferative response revealed a progressive decrease in cell numbers in autologous cultures (day 7: 42% initial no/well), a decrease largely attributable to death of the stimulator population.

A similar kinetic pattern was seen in heterologous cultures up to the fifth day, following which a phase of rapid cell division occurred, with a subsequent marked increase in cell numbers (Figure 1). This proliferative response was

![Figure 1. The effect of methylprednisolone on cell numbers in MLC: ● - - - autologous, ● - - - heterologous, ● - heterologous + MP 1 μg/ml](image)

inhibited by low concentrations of MP when present throughout the incubation (ID$_{50}$: 0.01 μg/ml), and was completely abolished by a dose of 1 μg/ml, as demonstrated by calculation of the percent proliferation. No significant difference in viability was seen between autologous cultures in the presence or absence of MP (0–10 μg/ml), thus mitigating against a cytolytic effect of this agent.

*Mixed Lymphocyte Culture*

A marked increase in $^3$H thymidine incorporation was seen in heterologous
cultures on the fifth day (59,395 ± 4428 cpm vs. 936 ± 157 control cpm). Suppression of this response was seen with 0.001 μg/ml MP, being essentially complete at 1–10 μg/ml (Figure 2). Some degree of inhibition of spontaneous \(^3\)H thymidine incorporation in autologous cultures was also seen in the presence of MP (0.001–10 μg/ml), but calculation of the Stimulation Index revealed a pattern of inhibition not significantly different from that expressed in cpm (61% suppression at 0.01 μg/ml, 93% suppression at 1 μg/ml).

**Effect of Methylprednisolone on the In Vitro Generation of Cytotoxic Effector Cells**

Cultures performed in the absence of MP resulted in the generation of specific cytotoxic killer cells (mean Sp \(^{51}\)Cr release: 27% in 4 hr and 61% in 16 hr LMC assay).

The presence of MP throughout the period of incubation resulted in marked or total suppression of killer-cell generation, inhibition being seen with a dose as low as 0.001 μg/ml and being complete, in most subjects, at 1.0 μg/ml. A considerable variation in individual sensitivity was noted (ID\(_{50}\): 0.0003 to 0.8 μg/ml, ID\(_{100}\): 0.01–10 μg/ml), subjects being either sensitive to or resistant to this agent. Cultures generated against differing stimulator cell populations demonstrated uniform responder-cell responses to MP, the resistance to suppression being directly related to cytotoxic capacity. The generation of effector cells in Falcon flasks, with equilibration of cell numbers prior to cytotoxicity testing, was similarly suppressed by MP when this agent was present throughout the period of incubation.

**Effect of Methylprednisolone on Lymphocyte-mediated Cytotoxicity**

Killer cells generated in vitro in the absence of MP exhibited specific cytotoxicity in both 4 and 16 hr assays, which was resistant to suppression by
Figure 3. The effect of methylprednisolone on killer cell generation in vitro (16 hr LMC assay)

Figure 4. The effect of methylprednisolone on the cytotoxic capacity of killer cells generated in vitro (4 hr LMC assay)
this agent at therapeutically achievable doses (0–20 μg/ml). In no instance was complete suppression achieved, although 50% inhibition was seen in two cases with a concentration of 5–10 μg/ml, after which no further suppression occurred. Methylprednisolone showed no cytolysis activity, as measured by $^{51}$Cr release from target cells, up to a concentration of 200 μg/ml, following which the spontaneous release increased progressively (Figures 3 and 4).

Discussion

Methylprednisolone is one of the major therapeutic agents currently employed in the field of renal allotransplantation, being used both to suppress the development of recipient anti-donor immune responses, and to abrogate established acute allograft rejection.

The individual variation in sensitivity to this agent at a clinical level may represent differences in pharmacokinetics [5] or in cellular distribution following administration [6], or more fundamentally, may represent physiological differences in response to this agent at a cellular level [7]. The development of lymphocyte-mediated cytotoxicity is a complex phenomenon. Following sensitisation by alloantigens, a primary subpopulation of lymphocytes undergoes proliferation, giving rise to cells with helper activity which facilitate the development of specifically cytotoxic T lymphocytes or killer-cells from a second population [8]. Inhibition of killer-cell generation, or suppression of their cytolytic capacity once formed thus represent fundamental mechanisms by which MP may effect modulation of cellular cytotoxicity in the transplant recipient.

Cellular proliferation, as measured by counting or $^3$H thymidine incorporation, was markedly inhibited or completely suppressed without cytolysis by the presence of MP during the sensitisation stage, thus representing an inhibition of the normal metabolic responses following antigenic stimulation. Cytotoxicity is markedly attenuated in the absence of helper-cell proliferation [9], and this may therefore represent one of the major mechanisms of MP mediated immunosuppression.

The generation of cytotoxic effector cells was similarly markedly inhibited or completely suppressed in all subjects by the presence of MP. This finding has been reported in steroid-sensitive species [10,11], but has been questioned in man [12].

The macro-culture studies performed with equilibration of effector cell numbers would suggest that this represents a true suppression of killer-cell generation, and is not simply attributable to numerical variations. A considerable individual variability in response to MP was noted, whether measured as ID$_{25}$, ID$_{75}$ (not shown) or as ID$_{50}$ or ID$_{100}$. The majority of patients were suppressible at therapeutically achievable doses, although some only at the peak serum level achieved by low dose oral prednisone, and in one case complete suppression was not obtained. This finding has since been confirmed on a larger population, and it would appear that the suppressibility of the subject is relatively constant and related to cytotoxic capacity. This suggests a direct metabolic competition between the processes of cytotoxicity and sup-
pression, and may be of fundamental importance in further understanding of these mechanisms.

Once generated in the absence of MP, killer cells remain refractory to suppression whether assayed at 4 or 16 hours. This is in keeping with other reports [13], but further studies of LMC generated in vivo in transplant recipients suggest that this may behave differently from primary cultures, killer cells exhibiting a dual response correlating with the clinical course (Keown et al - unpublished observations).

These preliminary studies have illuminated to some extent the complex mechanisms of MP action, and have demonstrated that variations in clinical response may be due not only to cell transport and pharmacokinetics, but also to a fundamental quantitative difference in response at a cellular level. Determination of such individual responsiveness prior to transplantation, in concert with a comprehensive post-transplant monitoring programme, may lead to a more effective application of therapeutic principles permitting optimum immunoregulation with the minimum adverse effect.

References


Open Discussion

STRUYVENBERG (Utrecht) Have you an explanation for the variability of the reaction to methylprednisolone of cells taken from different graft recipients?

KEOWN There are probably two or three explanations. One is a difference in receptor expression or affinity and the other the modulation of an intracellular mechanism which is post-receptor. We originally considered receptor expression or affinity to be the important factor, but in fact according to recent work performed by Dr Duval at Necker it looks as though these do not vary significantly. It may be activation of receptors, if receptors are involved. Failing that, the difference may lie in the modulation of the cyclic AMP system within the cell, either in the generation of cyclic AMP or in the regulation of phosphodiesterase which may be a point of competitive interaction between the two processes. Further than that I cannot explain at the moment.