ACCELERATED VASCULAR DISEASE FOLLOWING RENAL TRANSPLANTATION

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Introduction

Despite impressive technologic advances in the treatment of chronic renal failure, the long-term survival of both chronic dialysis1 and successfully transplanted renal patients2 is limited by an unexplained acceleration of cardiovascular disease. Indeed, the histological alterations found in atherosclerotic vessels from renal allograft recipients demonstrate three fundamental features common to advanced vascular disease: proliferation of arterial smooth muscle cells, deposition of intra- and extracellular lipid — mainly cholesterol and cholesterol ester — and the accumulation of extracellular matrix.3 The recognition that arterial smooth muscle cell (SMC) proliferation is an essential feature of all atherosclerotic lesions has stimulated the development of tissue culture techniques which now make it possible to identify factors which influence smooth muscle cell proliferation and the capacity of these cells to metabolise plasma lipoproteins.

We report the results of tissue culture studies undertaken to address the following questions related to the pathogenesis of accelerated cardiovascular disease in renal allograft patients:

Are humoral substances present in the serum of renal transplant patients which 1) accelerate the growth of SMC, and 2) alter the capacity of SMCs to metabolise low-density lipoprotein (LDL) — the cholesterol-rich lipoprotein class with the clearest association with premature cardiovascular disease?

Methods

Human arterial SMC were obtained from explants of renal artery removed from renal allograft recipients at the time of transplantation according to the method of Ross4, and grown in the Dulbecco-Vogt modification of Eagle’s medium. In these growth studies, cells were counted in quadruplicate and expressed as numbers of cells per plate. Cell counts were highly correlated with
the Lowry protein. Cells were grown in 10% human serum except where otherwise specified.

Results

Results of the first series of experiments (Figure 1) compare the growth promoting effects of serum from renal allograft recipients with normal renal function, steroid-treated asthmatics receiving an identical mean dose of prednisolone, and age plus sex matched controls. These data, shown on a logarithmic scale

![Graph showing effect of 10% serum from renal patients on arterial smooth muscle cell proliferation.](image)

which graphically reduces the differences between these responses, indicate that serum from both transplant and steroid-treated asthmatics caused a greater stimulation of SMC growth than serum from the control subjects. The differences observed between transplant and controls (p<0.001) and transplant and steroid groups (p<0.001) were statistically significant by the analysis of variance.

When these results and those obtained in four additional studies were expressed as a percentage of the control response on day 12, the concluding day of the experiment, a mean increment of 62% above control was observed (Figure 2). This stimulatory effect was statistically significant at the p<0.001 level. Therefore, serum from transplant patients caused a greater stimulation of SMC proliferation than serum from control subjects. In order to determine whether this growth-promoting effect was contained in the lipid or non-lipid fraction of plasma, transplant and control plasma were subjected to preparative ultracentrifugation and their non-lipid fractions of density greater than 1.25 g/dl were added to a similar test system containing 5% control serum.
EFFECT OF RENAL ALLOGRAFT SERUM (10%) ON ARTERIAL SMOOTH MUSCLE CELL GROWTH.

![Graph showing growth of cells with varying percentages of control over days]

\( \bar{x} = 62.4\% \)

\( p < 0.001 \)

Figure 2

EFFECT OF SERUM FRACTION (d > 1.25g/ml) ON ARTERIAL SMOOTH MUSCLE CELL PROLIFERATION.

![Graph showing proliferation of cells with transplant and control groups over days]

1% FCS

Figure 3
The growth response of the lipoprotein-poor plasma fraction from the transplant group was greater than control at all points on the curve and differed statistically at the p<0.01 level by analysis of variance (Figure 3). Expressed as a percentage of control, this specific response — and those observed in additional experiments with arterial smooth muscle cells revealed that the growth-promoting property of this fraction of transplant plasma was 25% greater than that observed in the plasma of controls.

This finding indicated that the ability of the lipoprotein-poor plasma fraction from transplant patients to stimulate SMC growth is increased, and this property contributes to the enhanced growth observed with whole serum.

The isotopic technique of Bierman, Stein, and Stein5 which provides an experimental means of estimating the three basic steps in LDL metabolism was employed to determine whether transplant plasma contains substances which alter the capacity of SMCs to metabolise low-density lipoprotein. With this method, the

1. Binding of LDL to the cell surface can be quantitated as the radioactivity released after brief exposure to trypsin;

2. The uptake or internalisation of the iodinated lipoprotein can be determined from the radioactivity counts in the cell pellet; and

3. The degradation of the internalised LDL may be estimated from the water-soluble radioactivity appearing in the medium. Since cholesterol accumulation is an integral feature of atherosclerotic lesions, factors which promote LDL binding and uptake and decrease degradation would theoretically be atherogenic.

![Effects of steroid and transplant serum on 125I-LDL binding & uptake](image1)

![125I-LDL degradation](image2)

Figure 4
Figure 4 compares the effects of lipoprotein-poor plasma from steroid, transplant, and control groups on these parameters of LDL metabolism over the 48 hour period of study. Transplant and steroid plasma caused an increase of both binding and uptake of the $^{125}$I-LDL – effects which must be considered potentially deleterious, if there is not a concomitant increase in the cells’ ability to degrade the internalised lipoprotein. No increase in degradation was observed, however. In fact, less degradation was observed in both transplant and steroid groups at all three sampling intervals. An identical pattern of $^{125}$I-LDL binding, uptake, and degradation was also found in studies in human dermal fibroblasts. The combination of increased binding and uptake and decreased degradation of LDL would favour the intracellular accumulation of lipid.

Discussion

Electron microscopic studies obtained by my collaborator, Dr Karen Holbrook, which show increased intracellular accumulation of lipid provide morphological evidence consistent with our incubation data, suggesting that transplant serum promotes the intracellular accumulation of lipid.

These studies indicate that serum from renal allograft recipients stimulates arterial smooth muscle cell proliferation. The lipid-free fraction of serum (d 1.25 g/ml) contains factors which contribute to this proliferative response, and also promote the binding and uptake and delay the degradation of $^{125}$I-LDL. These properties may influence the development of accelerated cardiovascular disease in renal allograft recipients.

References


Open Discussion

MASSRY (Los Angeles) Does uraemic serum have the same effect as serum from transplant patients?

BAGDADE Dr Massry has raised an obvious and an important question, namely, does serum from dialysis patients have a similar growth promoting effect on the smooth muscle cell system? Indeed it does. In this particular study the effect of uraemic serum was statistically significantly greater than control and less than transplant. However, with other pools of uraemic serum, I find the opposite relationship; that is, uraemic serum may actually cause greater cell proliferation than transplant serum. It is consistently greater than the control serum.

MASSRY Parathyroid hormone can induce growth of cells and proliferation
of cells in tissue culture, and I was wondering, since both uraemic and transplant serum do this, and probably both had high levels of parathyroid hormone, whether the obvious step is to see whether parathyroid hormone by itself has the same effect. I am sure you have thought of it. Do you have any data on that?

BAGDADE That is an excellent suggestion and it is a study that needs to be done.

BRYNGER (Gothenburg) At what stage after transplantation did you obtain the serum?

BAGDADE The minimum time was six months. Most patients were stable for more than a year and had normal renal function.

BRYNGER These data are very beautiful but they do not fit in with the experience of transplant surgeons. What will happen when you use muscle cells that have not been exposed to the uraemic state?

BAGDADE There is one point which I did not make clearly enough. The cells that we used in these studies which I have described are actually from renal allograft recipients. So these smooth muscle cells have been in a uraemic host. As a result there are certain questions and objections which might be raised on that basis. For this reason we performed identical studies in fibroblasts from dermal non-uraemic donors and the results were identical. Studies are now under way repeating the same studies in non-uraemic smooth muscle cells and they appear to respond identically. So I feel that the effects that we have observed are quite reproducible. I believe that these findings have particular relevance to the development of cardiovascular disease in these patients.

BRYNGER Yes, but what will happen after five years of good renal function? We have clinical experience in about 150 patients; we have been able to drop off all antihypertensive drugs, etc. We have been forced to operate on some of them after five years for different reasons. We know that they had vascular disease at the time of transplantation but after five years they were, microscopically, in a dramatically better state, and these data do not fit.

BAGDADE I am interested in your observation. I think that the aetiology of cardiovascular disease in any patient, whether an uraemic patient or a transplanted patient, is multifactorial. The co-existence of hypertension, hyperlipidaemia, smoking, possibly the duration of pre-existing uraemia, the age of the patient, the immunosuppressive regimen — all these factors are important in determining the survival of the patient, and the rate of development of cardiovascular disease.

HÄYRY (Helsinki) I wonder, how exclusive is the action of your serum factor on these smooth muscle cells? Did you by any chance check whether they also affect the growth of, let us say, human diploid cell lines or human tumour cell lines? I ask this because your factor which could possibly be an ordinary in vitro artefact, very often appear in these kind of studies.

BAGDADE That was the reason I mentioned that we specifically examined this question by using dermal fibroblasts and the biological effects were identical.