The role of cell proliferation and migration in the development of a neo-intimal layer in veins grafted into arteries, in rats

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Summary. The development of a thickened (hyperplastic) fibro-cellular neo-intima is a significant event in the adaptation of a vein grafted into an artery. The histogenesis of tissues in vein grafts was explored in a rat model where the source of endothelial and smooth muscle cells was from the adjacent artery. Cell proliferation was assessed by the incorporation of tritiated thymidine and autoradiography, up to 18 months after grafting. Cell migration was detected by prelabelling in the first 5 days after grafting and sampling at later times. The proliferation of cells in the arterial media adjacent to the graft was elevated above control levels as early as 2 days after grafting; it was maximal at 3 days and returned to low levels by day 21. During the first week, prelabelled smooth muscle cells in the tunica media of the adjacent artery migrated to the subendothelial space, where they continued to proliferate to produce arterial intimal hyperplasia. The migration of endothelial and smooth muscle cells proceeded across the anastomosis to populate the vein graft neo-intima, where smooth muscle cells continued to proliferate until 28 days after grafting. Cell migration and proliferation were significant factors in the histogenesis of vein graft neo-intimal hyperplasia in this model. These processes were controlled, perhaps by local regulatory factors, to form a vein graft, the wall of which was similar in thickness and structure to that of the host artery.

Key words: Vein grafts – Intimal hyperplasia – Cell proliferation – Smooth muscle – Endothelium – Autoradiography – Rats (Wistar)

When a vein segment is inserted into an artery the vein undergoes a histological transformation into a pseudo-artery. Most of the change occurs in the tunica intima of the vein segment, where smooth muscle cells (SMC) invade the sub-endothelial region to produce what is commonly termed the “neo-intima”. (This process is also known as neo-intimal hyperplasia.) The region is similar functionally and morphologically to the tunica media of an artery. The present study was designed to investigate the histogenesis of cells in the neo-intima of vein grafts, both as a model to determine the cellular biological changes that occur in vascular tissues, and also to understand the adaptive changes in clinical vein-to-artery grafts.

Previous morphological studies have shown that fibrocellular neo-intimal hyperplasia is the most important change in a vein graft (Szilagyi et al. 1973; Fuchs et al. 1978; Campbell et al. 1981; Dobrin et al. 1988; McGeachie et al. 1989). Migration and proliferation of SMC play a major role in neo-intimal hyperplasia (Spaet et al. 1975; Clowes and Schwartz 1985; Tennant et al. 1990), but these processes are rarely observed in the normal vessel wall. Arterial intimal hyperplasia develops when cell proliferation and migration are significantly increased after trauma, such as suture placement or endothelial denudation (Webster et al. 1974; Baumgartner and Studer 1978; Clowes et al. 1983; Clowes and Clowes 1985; Liu et al. 1989). The surgical trauma which occurs during insertion of a vein graft is an obvious stimulus for increasing cell proliferation. Experimental models of vein grafting have provided the most information on the histogenesis of neo-intimal hyperplasia. However, there is only scant evidence on the role of cell proliferation in this process, as shown for example by mitotic figures (Brody et al. 1972) or tritiated thymidine (3H-TdR) uptake (Zwolach et al. 1987).

In a rat model, in which a 4 mm segment of the ilio-lumbar vein is inserted into the iliac artery (1 mm in diameter), the graft neo-intima develops by ingrowth of cells from the adjacent arterial media (Dilley et al. 1986, 1988). In the present study we describe the role of cell proliferation and migration in the histogenesis of neo-intimal hyperplasia. Cell proliferation was measured by 3H-TdR incorporation and autoradiography. The incorporation of 3H-TdR is rapid (Diderholm et al. 1962; Chang and Looney 1965; Cleaver 1967), and once
incorporated is very stable and will remain for the life of the cell and its progeny (Cleaver 1967). The short availability time of \(^3\)H-TdR after injection (30-60 min) and its long term stability ensure that labelling which occurs prior to the commencement of neo-intimal hyperplasia will remain after the subsequent migration of labelled cells.

Materials and methods

Cell proliferation (pre-mitotic labelling)

Iliolumbar vein to iliac artery grafts (Prendergast et al. 1979; McGeachie et al. 1981) were performed on 27 male albino Wistar rats (248 ± 8 g, mean weight ± SEM) under Nembutal (pentobarbitone) anaesthesia. One animal was sacrificed for each of eleven time groups (1, 2, 3, 5, 7, 14, 21, 28, 124, 365, 547 days) and then four more animals at each significant time in graft neo-intimal development (3, 5, 14 and 28 days). One hour prior to sacrifice each rat received a single i.p. injection (1 μCi/g body weight) of \(^3\)H-TdR (5 Ci/m mole, Amersham). All labelled cells were premitotic at the time of graft removal.

Cell migration (post-mitotic labelling)

Twelve additional rats (239 ± 6 g) received grafts and a single intra-peritoneal injection of \(^3\)H-TdR at 2 or 5 days after grafting, as described above. Two rats per group were sacrificed either at 1 h after injection (to measure base-line premitotic labelling levels), 5 days after injection, or 14 days after grafting; thus the labelled cells in the latter two groups were post-mitotic when sampled.

Tissue preparation

All animals were fixed by intra-cardiac perfusion with a mixture of 2.5% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) at physiological pressure. Tissues were taken from the graft, adjacent artery, the opposite iliac artery and intestine, block-stained with p-phenylene-diamine and embedded in Epon/Araldite, as previously described (Dilley and McGeachie 1983). Four longitudinal sections from each graft, along with sections of the artery adjacent to the graft, and the control artery from the opposite (unoperated) side, and intestine, were coated with Kodak AR10 stripping film and autoradiographed (Dilley and McGeachie 1983). The percentages of SMC and endothelial cells which were labelled (labelling indices) were determined for 1 mm either side of the anastomosis. A cell having 4 or more grains per nucleus was considered to be labelled.

Because percentages of labelled cells were recorded, arcsin transformations of the data were used to normalise the distribution for statistical analysis. Data were tested by analysis of variance (ANOVA).

Results

Cell proliferation (pre-mitotic labelling)

Arterial controls. Very few cells were labelled with \(^3\)H-TdR in the opposite control iliac arteries. A total population of 3748 medial SMC were counted from control iliac artery sections and only two cells (0.05%) were labelled.

Grafts and adjacent arteries. Examples of labelled SMC are shown in Fig. 1. In the adjacent artery, there were no labelled cells at 1 day, but heavier labelling than in controls (0.43% compared with 0.05%) was first observed in SMC of the arterial media at 2 days after grafting. There were no sub-endothelial cells in the adjacent artery, and no endothelial cells in the graft neo-intima at 2 days. Three days after grafting, cell labelling in the adjacent arterial media had increased to a peak level of 8.2% (Table 1). Also at 3 days cellular infiltration into the sub-endothelial space (intimal hyperplasia) had commenced in some adjacent arteries, and about one in every four of the sub-endothelial cells were labelled at much higher levels than in media (Table 1). Endothelial cells were also heavily labelled in the adjacent arteries at 3 days (Table 1) and re-endothelialisation had progressed towards the anastomosis. By 5 days, the levels of labelling had declined in the adjacent arterial endothelial cells and medial SMC (Table 1), but remained much higher than in control iliac arteries in the same animals. In the graft at 5 days, neo-intimal cells (endothelium and subendothelial cells) were few but showed high levels of labelling (Table 1).

Fourteen days after grafting, labelling levels in the adjacent artery were low, but remained relatively high in the graft (Table 1). Neo-intimal cell labelling at the

Fig. 1 a, b. Labelling of smooth muscle cells in the vein graft neo-intima at 28 days after grafting. Tissue samples were removed 1 h after \(^3\)H-TdR injection, therefore these cells are pre-mitotic; a is focussed on the cells and b focussed on the autoradiographic grains (La). The arrow indicates the original inner elastic lamina of the vein. L Vessel lumen