

Is *Phaseolus vulgaris* Leucoagglutinin (PHA-L) a Useful Marker for Labeling Neural Grafts?

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ABSTRACT

The lectin *Phaseolus vulgaris* leucoagglutinin (PHA-L) has come into wide use as an antero-grade neuroanatomical tracer. The ability of this lectin to fill entire neurons and remain in place over long periods suggested it might be an ideal marker for donor cells to be grafted into hosts for long survival periods. We have used the lectin PHA-L to mark fetal rat olfactory bulb (OB) cells prior to grafting into host rat OBs. Hosts were sacrificed at various times up to 9 weeks after grafting, and tissue was immunohistochemically processed for PHA reactivity. After 2 and 4 weeks survival, sparse patterns of labeled cells were observed within the host OBs. However, after 9 weeks survival, few if any labeled cells were visible within host tissue. We conclude that PHA-L may be a less than satisfactory marker for fetal rat cells (other than astrocytes) which are to be identified in host tissue after a period of several weeks.

Key words: Transplantation; grafting; fetal; donor; host; *Phaseolus vulgaris* leucoagglutinin (PHA-L); olfactory system

In neural grafting paradigms involving replacement of host tissue with homotypic donor cells, results are often ambiguous unless fetal cells are in some way marked prior to grafting. Several different marking methods, including horse-radish peroxidase (HRP), fluorescent dyes, and [³H]thymidine, have been used with varying degrees of success /5,6,8,9,12/. However, HRP and fluorescent dyes may be too short-lived within neurons for many experiments /8,9,11/ and [³H]thymidine, which only labels nuclei, is incorporated only at the time of cell division or DNA repair. *Phaseolus vulgaris* leucoagglutinin (PHA-L), a lectin commonly used as an antero-grade neuro-anatomical tracer, is longer-lived than HRP within cells /4/.

Previous reports suggest that this lectin may (under certain conditions) be a useful marker for donor cells. Specifically, PHA-L has been used to mark cultured astrocytes /5,6/ and cultured human spinal cord cells transplanted into rat motor cortex /7/. In a short-term study (5-21 days survival), PHA-L has been used to mark suspensions of fetal rat brain subsequently grafted into various areas of adult rat brains /10/. However, our experience, as well as that of other researchers with whom we have communicated (Kunkel and Schwartzkroin, unpublished observations; Raisman and Field, personal communication), suggests that PHA-L is a less than satisfactory long-term label for fetal rat neurons. These findings are presented in the hope that our experience may prevent unnecessary duplication of effort.

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Approximately 20 fetal rat olfactory bulbs (OBs) (E18; crown-rump length = 22-24 mm) were removed, trypsinized, and dissociated in glucose enhanced saline solution /2/. Following dissociation, cells were incubated in the same medium with 0.5% PHA-L for 15 minutes. These incubation parameters were similar to those used for cultured human spinal cord cells /7/ (1% PHA-L for 10 min.) and produced strong initial labeling of donor cells (Fig. 1). To remove as much unbound PHAL as possible, labeled cells were washed 4 times by adding at least 20 volumes of medium followed by mild centrifugation and removal (with a pipette) of excess medium. Host animals were 5-day-old neonates from a single litter of the same strain as the donors. Immediately following preparation of donor cells, grafting was performed under ether anesthesia. The dorsal surface of the right OB was exposed using standard surgical technique. Eight animals each received an injection of approximately 12 μ l of cell suspension slowly delivered into the core of the right OB using a drawn glass injector pipette (approx. 400 μ m O.D.) attached to a 50 μ l syringe by a length of PE-100 tubing. Three animals received control injections of a similar volume of medium only. Injections were manually delivered over a period of 20-30 seconds. In order to help estimate the completeness of labeling, a similar volume of labeled cells was placed in the dorsal cortex of an adult host for *in vivo* 24-hour incubation. The adult cortex, rather than the OB, was selected for this examination because of ready access and greater mechanical stability during processing. To assess the number of cells per injection, 12 μ l portions of the cell suspension were placed on gelatinized slides, fixed, and stained with cresyl violet for immediate microscopic examination.

After survival of 2 and 4 weeks, groups composed of 3 animals with grafts and 1 control animal were sacrificed by transcardial perfusion under deep anesthesia with 4% paraformaldehyde in 0.1 M phosphate buffer. The remaining 2 animals with grafts and single control were similarly sacrificed after 9 weeks survival. After 1 hour postfixation in the above solution, brains were removed and cryoprotected in phosphate buffered 30% sucrose for at least one day. Subsequently, 33 μ m sagittal sections were cut on a

freezing microtome and processed for PHA reactivity using Vector ABC reagents for immunoperoxidase /4/. After a 24-hour *in vivo* incubation period the brain of the single adult host was prepared as above except that 8 μ m cryostat sections were processed for PHA reactivity on slides. In all cases, alternate control sections were processed without the primary antibody.

Cell counts made from micrographs of the 12 μ l portions of cell suspension applied to slides indicated that each portion contained at least several thousand cells.

After 24 hours of *in vivo* incubation (within the adult cortex) a large number of apparently viable and heavily labeled cells were found "clumped" within the wound channel left by the injection cannula. While most of these cells were well localized within the wound channel and were usually attached to its sides, occasional PHA reactive cells were found within what appeared to be undisturbed adjacent host tissue (Fig.1).

After 2 and 4 weeks survival times a few labeled cells were seen in the host OBs (Fig. 2). While the number of animals in each group was too small to allow a meaningful quantitative comparison of the number of labeled cells present after 2 and 4 weeks, the number and pattern of labeled cells were qualitatively similar at the two survival times. At both survival times the number of cells exhibiting reactivity appeared quite low compared with the original number of cells injected. By 9 weeks post-grafting, only a small number of reactive cells could be seen in one brain and none at all in the other. The labeling that was present in the first 9 week survival brain was considerably less dense than that in the 2- and 4-week preparations.

We have attempted to evaluate the applicability of PHA-L as a marker for a dissociated mixture of cell types grafted into a homotypic host area. When evaluating any marker for donor cells there are several issues to be considered: 1) proportion of cells initially labeled; 2) proportion of labeled cells surviving for a useful time period; 3) survival of marker within labeled cells; 4) dilution of marker through cell division; 5) transfer of marker from donor to host cells (either transynaptically or from labeled nonviable donor cells); 6) possible

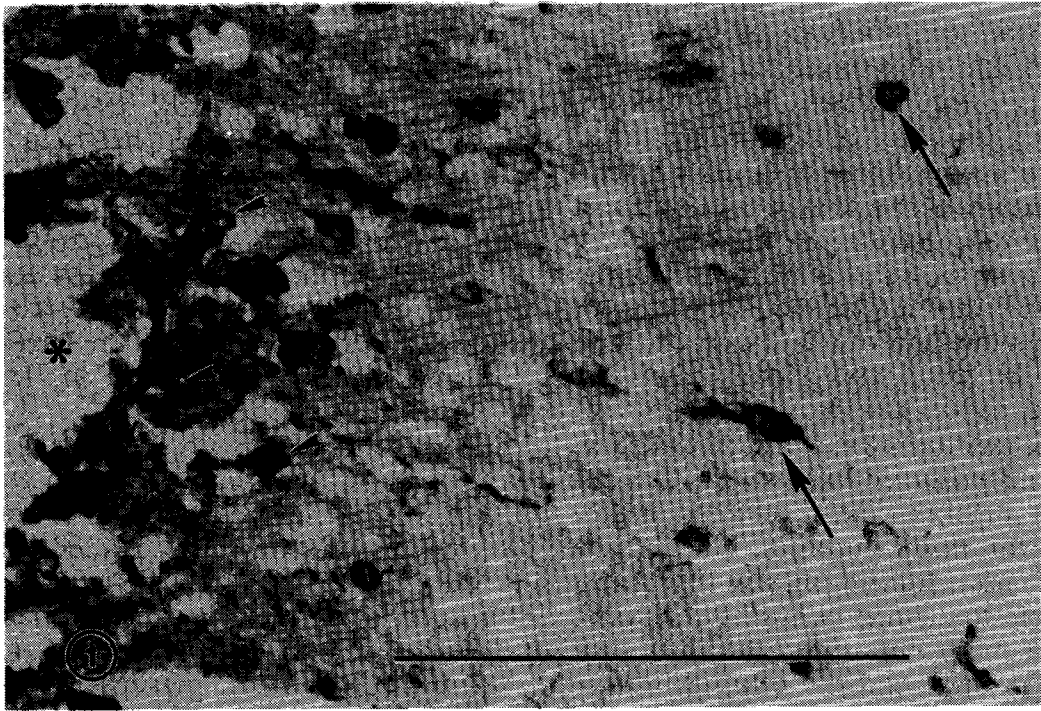


Fig. 1: Uncounterstained cryostat section from deep within adult host cortex into which PHA-L labeled cell suspension was injected 24 hours earlier. Heavily labeled cells (arrow heads for example) along wound channel (*) are presumed to be of donor origin, but note labeled cells (arrows) in host cortex. Bar = 100 μm .

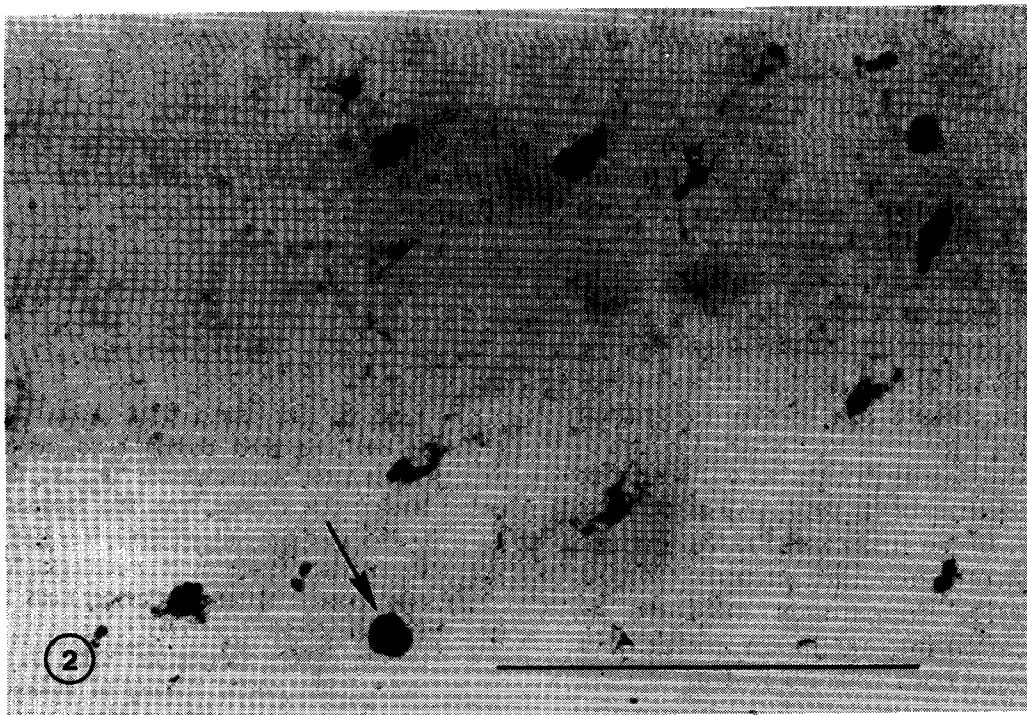


Fig. 2: Sparse pattern of labeled cells in a 33 μm noncounterstained frozen section after a 4-week survival period. Some macrophages (arrow) are still present at this stage. Bar = 100 μm .

migration of labeled donor cells away from the graft site. Finally, one must be reasonably assured that unincorporated label can be separated from the donor cells prior to grafting. Obviously, if a marker does not strongly label a significant portion of the donor cells to begin with, the other issues are difficult to address.

In some situations /e.g., see ref. 5,6/ where the aim is to show the unquantified migration of astrocytes from one part of the CNS to another, PHA-L may be quite appropriate. However, while it would appear that most cells which we originally exposed to this lectin are labeled (Fig. 1), the low yield of labeled cells (even at 2 weeks post-grafting) makes this method highly questionable for many purposes. Such a low proportion of cells containing marker is probably not attributable to dilution of label through cell division, since many had undergone their final division at the stage that the fetal cells were taken /3/. Further, in support of a previous report /7/, we found little evidence for extensive migration of labeled cells. Therefore, our low yield of labeled cells may be explained by either of at least two mechanisms acting singly or in combination, i.e., PHA-L may have been degraded within cells or many labeled cells may not survive for as long as 2 weeks. It is possible that the few labeled cells seen in host tissue adjacent to the injection site in our 24 hour *in vivo* preparation were undergoing migration, and it is possible that migration could explain a small percentage of the labeled cells lost from the graft site. However, it is equally possible that these are host cells labeled by PHA-L either remaining in the injection medium or released from nonviable donor cells. This potentially troublesome finding should be addressed in any future studies attempting to use PHA-L as a marker for grafted cells.

The successful use of PHA-L as a marker for neuron-like cells has been reported in at least two cases/7,10/. In the first case, PHA-L labeled human spinal cord material, cultured for an unspecified period (apparently measured in weeks), was grafted into rat motor cortex. It is unclear why spinal cord cells containing PHA-L should survive and retain the label more readily than cells of the OB. It is possible that, prior to labeling, the cultured spinal cord cells may have reached a greater state of maturity and

differentiation than our OB cells. Perhaps the loss or retention of PHA reactivity is related to differences in metabolic activity of cells in varying stages of development. In the second case /10/, studies indicated that PHA-L labeled donor cells are readily demonstrated within the host brain after relatively short (5-21 day) survival periods. These studies indicate that lectin toxicity may not be a problem in cell survival but do not address the issue of the final disposition of the lectin in longer term experiments.

In confirmation of the findings presented here, PHA-L has proven to be an unsatisfactory marker for fetal donor cells in several other instances: OB fragments grafted into host OB (our laboratory, unpublished); striatal cell suspensions grafted into hippocampal locations (Kunkel and Schwartzkroin, unpublished; Raisman and Field, personal communication). This report is presented in the hope that it may prevent duplication of effort that might be better directed elsewhere.

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