Injury by Human Complement Causes Large Membrane Lesions that Reseal in IL-4-treated Porcine Endothelial Cells

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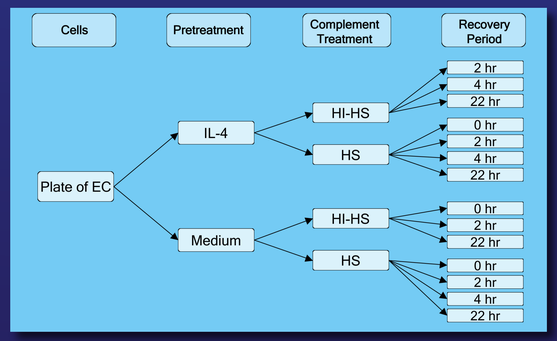
COMPLEMENT SYSTEM IMPORTANCE IN XENOTRANSPLANT REJECTION

- Interspecies organ transplantation (xenotransplantation) is unsuccessful due to the rejection of the organ by the recipient's innate immune system
- Xenotransplantation could be a solution to shortage of human organ donors
- The vascular endothelium of the transplant is the main target of injury by the host immune system, especially complement activation
- Complement system activation creates lesions in the membranes of foreign cells, leading to cell death
- The cytokine interleukin-4 (IL-4) is known to induce protection of endothelial cells (EC) from killing by complement in human serum (HS) (Ref. 2)
- This protection does not interfere with an initial membrane permeability alteration, suggesting that IL-4 causes protection through a membrane repair process (Ref. 1)

Hypothesis: The IL-4-protected cells, when exposed to complement, undergo a membrane lesion of defined size, and the repair process results in the complete structural and functional recovery of the cells.

MATERIALS AND METHODS

Treatment of EC with IL-4 and Complement. During the IL-4 pretreatment, EC were incubated with either medium alone (1% FBS/DMEM) or with medium containing 5 ng/ml IL-4 for 48 hrs. The EC were then incubated with solutions containing either 20% human serum (HS) as a source of antibody and complement or 20% heat-inactivated human serum (HI-HS) for 2 hrs. Complement solutions were then replaced with medium (1% FBS/DMEM) for a recovery period of 0, 2, 4 or 22 hrs.



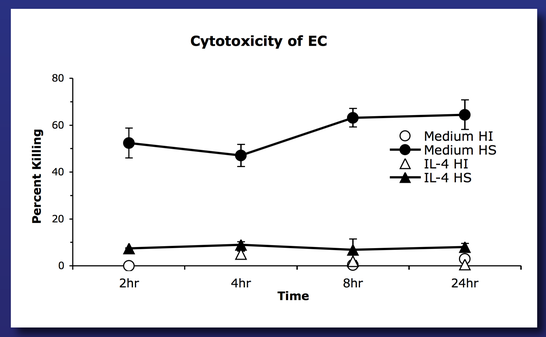
MATERIALS AND METHODS

Measurement of Complement Killing and Membrane Leakiness. EC cytotoxicity was measured by a vital dye assay using Neutral Red (NR) (Ref. 3). To measure membrane leakiness, a lactate dehydrogenase (LDH) assay was used. This measured the percentage of the cells' total LDH that had leaked into the supernatants. The EC themselves were used in the NR assay.

Visualization of Membrane Leakiness with Fluorescent Microscopy. EC were additionally incubated with propidium iodide (PI) during a 30 min complement treatment. No recovery period was included. All nuclei were stained with DAPI, then visualized under a microscope. Uptake of PI, a DNA intercalating agent, indicated the presence of membrane lesions during incubation.

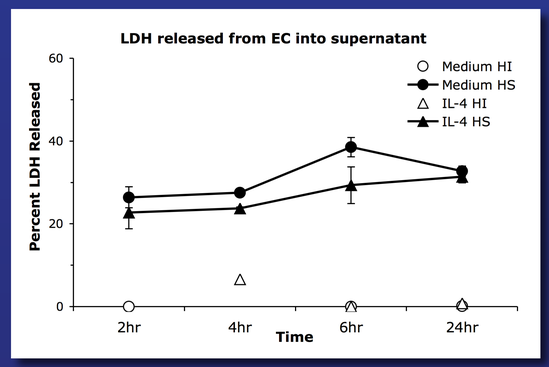
RESULTS

IL-4 induces protection of EC from complement killing, but LDH still leaks out of EC



EC killing as calculated by NR uptake was under 10% for all samples treated with HI-HS, regardless of IL-4 pretreatment. Treatment with active complement after IL-4 pretreatment (IL-4 HS) resulted in significantly less EC killing than treatment with complement and no IL-4 (Medium HS).

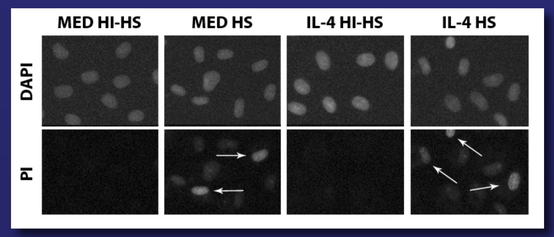
RESULTS (cont'd)



LDH release is a total of the 2 hr treatment and recovery time combined. Because the supernatant was removed and replaced with new medium after the 2 hr treatment, the 2 hr samples for each respective treatment were used as the baseline values for the other time points.

EC treated with active complement in HS leaked similar amounts of LDH into the supernatant, with or without being pre-incubated with IL-4. Less than 10% of LDH leaked out of all EC samples treated with HI-HS, regardless of IL-4 pretreatment.

IL-4 does not prevent PI uptake during complement treatment



DAPI uniformly stained all EC nuclei, alive or dead, whereas only EC with compromised, porous membranes during complement treatment were able to uptake PI. Treatment of PAEC with HI-HS resulted in almost no PI uptake, regardless of IL-4 pretreatment. Treatment of EC with active complement in HS resulted in significant numbers of EC containing PI. The numbers were similar regardless of pretreatment with IL-4 or just medium (MED). Arrows indicate cells that are defined as having lost permeability control to PI.

CONCLUSIONS

- Based on NR results, significant LDH loss from EC would not be expected in IL-4 protected cells. However, similar amounts of LDH are lost from cells treated with human complement (in HS) regardless of whether they were pretreated with IL-4 or not.
- Thus, IL-4 does not prevent the formation of lesions in EC during incubation with complement, but does help protect the cells from killing by complement. **IL-4-protected EC appear to have initially leaky membranes, but are able to reseal the membrane lesions caused by complement and avoid cell death.**
- IL-4-protected EC reseal membrane lesions during the first 2 hrs and stop releasing LDH after complement treatment during additional recovery period (incubation in medium) lasting up to 22 hrs.
- Similar PI uptake in both IL-4-protected and unprotected EC treated with complement further supports these conclusions.
- These findings support previous experiments studying calcein release in response to complement treatment (Ref. 1). In contrast to LDH, calcein is a very small molecule.
- LDH is a tetrameric protein of ~140 kDa, which suggests that the size of the lesions formed by complement must be relatively large. The maintenance of cell viability during the time that EC are leaky may be due to IL-4-induced metabolic changes that result in mitochondrial protection (Ref. 1).

Future directions: In order to determine the size of the lesions formed by complement, fluorescently labeled dextrans of different sizes will be incubated with EC during complement treatment. The sizes of dextrans taken up by treated and untreated cells will be analyzed to determine exact lesion size.

REFERENCES

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