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Lung transplantation: pulmonary cell lysis mediated by alveolar mononuclear cells

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Abstract. Methods were developed to monitor graft rejection in a porcine model of unilateral lung transplantation. The ability of peripheral blood mononuclear cells and lavage-derived mononuclear cells to lyse donor pulmonary tissue was determined by standard chromium release assays at various times after transplantation. Effective anti-graft activity was observed in the local environment of a rejecting graft, but not in the periphery. Since transplant rejection is a reversible process, with the administration of suitable immunosuppressive regimes frequently restoring graft function, it was reasoned that immunological assays based on the lysis of individual cells may not be relevant to the in vivo situation. We therefore describe an assay of the lung barrier function; perturbations of the tight intraepithelial junctions which compose the air-blood barrier can be determined in vitro by the measurement of transmonolayer resistance values.

Key words: Graft monitoring – Lung transplantation – Alveolar epithelium – Tissue resistance

Monitoring a transplanted organ is essential for the early diagnosis and effective treatment of rejection. Lung grafts can be physically observed by radiography or transbronchial biopsy [1, 2], their function can be monitored or immunological assays can be performed to determine the responsiveness of the recipient's immune system to the donor tissue.

A great deal of interest has been generated in the possibility of using bronchial lavage fluid for the diagnosis of lung allograft rejection. It has been shown that the mononuclear cells present in lavage fluid are recruited from the periphery, that the frequencies of donor-specific cytotoxic T lymphocytes in both the periphery and the local environment of a rejecting rat lung allograft increase after transplantation, and that mononuclear cells present within a lavage are effectively cytotoxic against donor but not third-party splenic lymphocytes [3–5]. However, it is also clear that the response of the immune system to a foreign graft can often appear similar to that mounted to a pathogen, and consequently great care must be taken in the interpretation of the results.

There are clear advantages in the use of donor graft tissues rather than donor splenic cells for the measurement of recipient anti-donor immunoreactivity. This approach allows responses to both donor tissue-specific and major histocompatibility antigens to be quantified. In this study, we investigated the cytotoxic ability of lavage-derived mononuclear cells (LDMC) and peripheral blood mononuclear cells (PBMC) against donor pulmonary cells in a porcine model of unilateral lung transplantation.

Since acute rejection is frequently a reversible process, immunological assays which measure the irreversible cytolysis of donor cells may not be applicable to in vitro investigation of the clinical situation. We report an assay of pulmonary tissue function which makes use of the fact that the tight junctions between alveolar epithelial cells can be measured in vitro by the determination of transmonolayer electrical resistance values. The interepithelial cell tight junctions are largely responsible for maintaining the blood-air permeability barrier essential for normal lung function [6, 7].

Materials and methods

Single lung transplants were performed in Gottingen minipigs (30–40 kg; Froxfield Farms, Hants, UK). Heart-lung blocks were retrieved from donors following perfusion with modified Euro-Collins solution; the left lung was dissected from the block and transplanted into the recipient (mean ischaemic time of 220 min, $n = 4$). Animals were immunosuppressed with azathioprine, cyclosporin A and prednisolone. The right lung was immersed into RPMI 1640 medium (Northumbria Biologicals, Northumberland, UK) on ice. Within 4 h a 2-cm^3 portion of the tissue was chopped and digested overnight by stirring with collagenase (Sigma, Dorset, UK) at 1 mg/ml (w/v) in RPMI 1640 (Northumbria Biologicals). After digestion, the material was washed twice by centrifugation and propagated in D-valine minimal essential medium (Gibco, Renfrewshire, UK) supplemented with 15% (v/v) heat-inactivated fetal calf serum (FCS),

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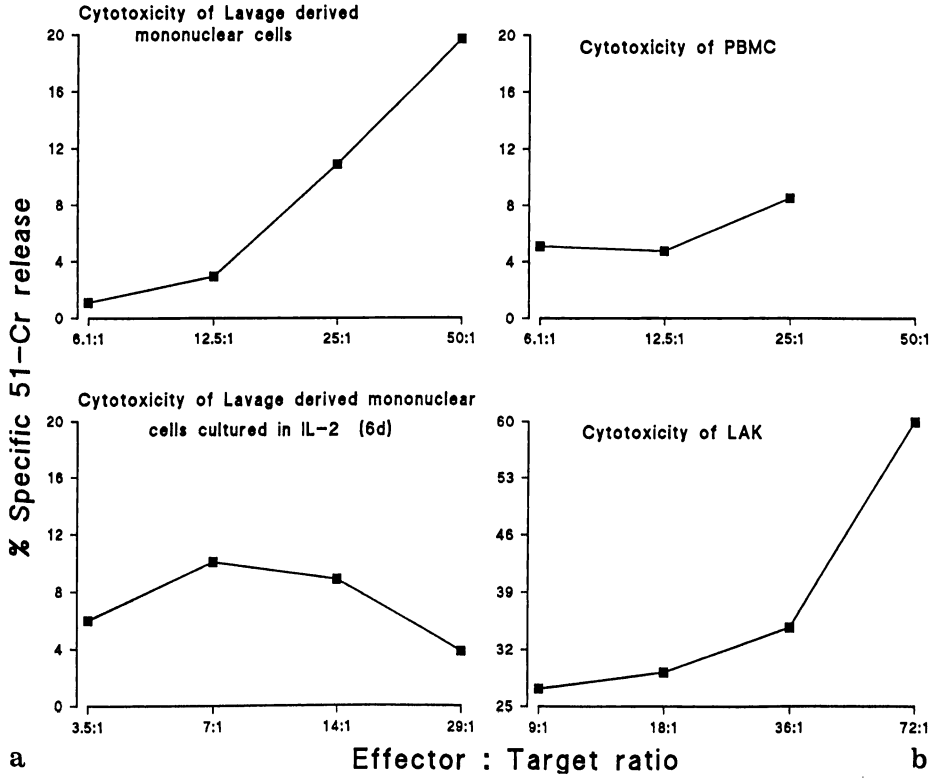

Fig. 1. Specific cytotoxicity of lavage-derived mononuclear cells (LDMC) and peripheral blood mononuclear cells (PBMC) towards pulmonary cells 1 week after transplantation. Each point represents the mean of triplicate determinations. *LAK*, lymphokine-activated killer cells; *IL-2*, interleukin 2

1×10^{-4} *M* HEPES (Northumbria Biologicals), 2×10^{-3} *M* glutamine (Northumbria Biologicals), 10^5 U/l penicillin (Sigma), 100 mg/l streptomycin (Sigma), insulin-transferrin-sodium selenite (Sigma), 5×10^{-8} *M* hydrocortisone (Sigma) and 3×10^{-8} *M* triiodothyronine (Sigma) at 37 °C in an atmosphere of 5% CO_2. Cells with a cobblestone morphology were purified by differential detachment using trypsin-ethylene diamine tetra-acetic acid (Northumbria Biologicals). Cell monolayers were cultured on 10-mm diameter tissue culture inserts (Nunc; Gibco, Renfrewshire, UK) by seeding each insert with 5×10^5 cells in 0.5 ml of the D-valine culture medium. Trans-monolayer resistance values were measured using an ohmmeter and 'chopstick' electrodes (Millicell-ERS, Millipore), as described previously [8].

Samples of peripheral blood (collected in sterile universals containing 100 U of heparin; Sigma) and bronchial lavage fluid (obtained by washing with approximately 20 ml RPMI 1640 medium through a fibreoptic bronchoscope wedged into a limiting bronchus of an anaesthetized recipient) were taken from recipient animals at regular intervals after transplantation. PBMC were prepared by centrifugation over a Ficoll-Metrizoate (400 *g*; Lymphoprep; Nycomed, W. Midlands, UK) density gradient [9]; the interfacial cells were recovered, washed and resuspended in RPMI 1640 culture medium supplemented with 10% (v/v) heat-inactivated FCS, 1×10^{-4} *M* HEPES and antibiotics. Lymphokine-activated killer (LAK) cells were generated from these by incubation in culture medium supplemented with recombinant interleukin 2 (IL-2) (50 U/ml; Boehringer Mannheim, East Sussex, UK) for 5 days. LDMC were obtained by centrifugation (400 *g*). Adherent cells (presumably alveolar macrophages) were removed by resuspending the cell pellet in culture medium and incubating in 25-cm^3 flasks (Falcon) at 37 °C in a humid atmosphere of 95% air and 5% CO_2 for at least 60 min. Donor pulmonary cells were labelled with 200 μCi of $Na_2{}^{51}CrO_4$ for 90 min, washed twice with RPMI 1640 and once with culture medium, resuspended at 2×10^4/ml and used as the targets in cytotoxicity assays. Effector cells (PBMC, LAK, LDMC or LDMC cultured in 50 U/ml IL-2 for 5 days) were incubated with the chromium-labelled pulmonary target cells at various effector: target ratios for 4 h at 37 °C in round-bottomed microtitre plates (Nunc) in a total volume of 200 μl. The plates were then centrifuged for 5 min at 50 *g* and the supernatants harvested for γ-counting (LKB, 1272 Clinnigamma). Maximal chromium release was estimated by lysing the target cells with 10 μl of Triton X-100 followed by freezing and thawing the samples. The cytolytic capacity of each culture was expressed in terms of percentage specific release of ^{51}Cr [10]:

$$\% \text{ specific } {}^{51}Cr \text{ release} = \frac{(\text{experimental } {}^{51}Cr \text{ release} - \text{spontaneous } {}^{51}Cr \text{ release})}{\text{maximal } {}^{51}Cr \text{ release} - \text{spontaneous } {}^{51}Cr \text{ releaseright})} \times 100$$

The effects of incubating 2×10^6 LAK or unstimulated porcine splenic cells (in 0.5 ml of culture medium) on the trans-monolayer resistance values of pulmonary cell monolayers cultured on tissue inserts was also determined.

Results

It was demonstrated that LDMC from an animal undergoing rejection were effectively cytotoxic against donor pulmonary cells, whereas PBMC were not. After culture in IL-2 for 5 days, PBMC were capable of lysing the donor pulmonary cells, presumably due to non-specific LAK function. The LDMC were not cytotoxic following culture in IL-2; this may have been due to the inhibitory effects of any pulmonary surfactant or alveolar macrophages remaining in the culture. The results presented in Fig. 1 are from an animal 7 days after transplantation; partial consolidation of the transplanted lung was observed by radiography at that time. One week later, it was demonstrated that the LDMC caused an increase in the maximum specific chromium release liberated from donor pulmonary cells; it had increased from 20% (Fig. 1) to 40% (Fig. 2b). However, LDMC from the native lung were also effective-

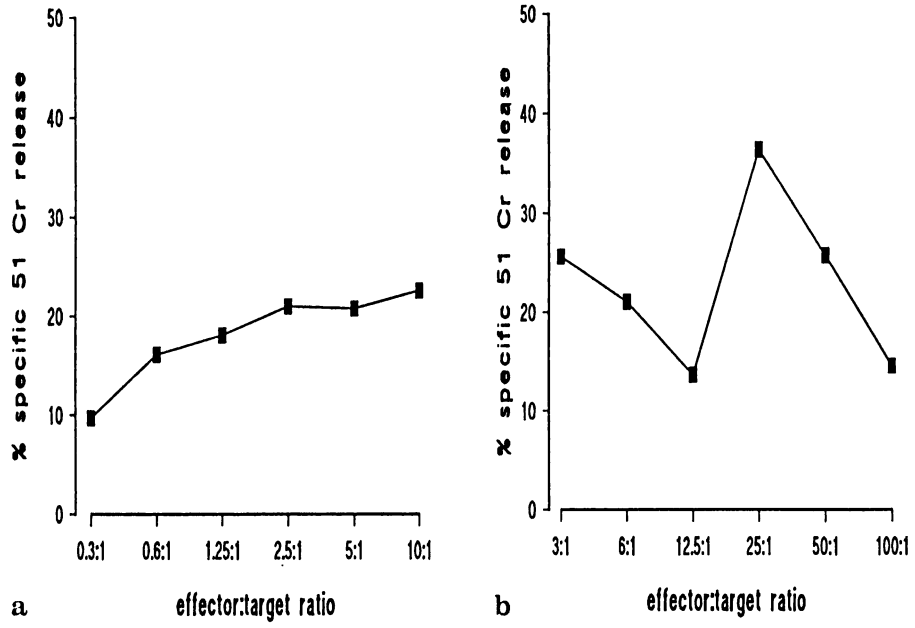

Fig. 2. **a** Specific cytotoxicity of LDMC from the native lung 2 weeks after transplantation. **b** Specific cytotoxicity of LDMC from the allograft 2 weeks post transplant. Each point represents the mean of triplicate determinations

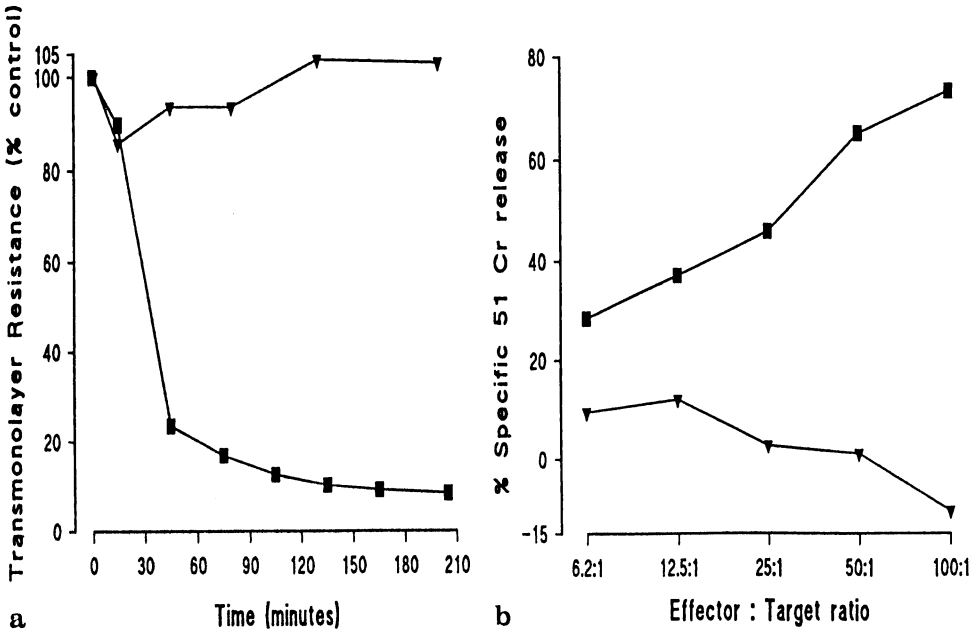

Fig. 3. **a** Representative results showing the effect on transmonolayer resistance values of adding 2×10^6 LAK (■) or porcine splenic cells (▼) to confluent porcine pulmonary cells. **b** Representative results showing the cytotoxicity of LAK cells (■) or splenic mononuclear cells (▼) towards porcine pulmonary cells. Each point represents the mean of triplicate determinations

ly cytotoxic against the donor pulmonary cells (Fig. 2a); subsequent investigation showed that the animal had developed bacterial pneumonia.

Porcine pulmonary cells cultured on inserts developed high trans-monolayer resistance values which generally reached a plateau on the 5th day of culture. At this time, the average value was 5500 Ω. The fact that primary porcine pulmonary cell monolayers produced high trans-monolayer resistance values provides good evidence that this cell population retains epithelioid characteristics.

The addition of LAK cells to the monolayers at an approximate effector: target cell radio of 4:1 caused an 80% reduction in the trans-monolayer resistance values within a 60-min period (Fig. 3a). Control experiments performed with unstimulated splenic mononuclear cells showed no change in resistance. In a standard 4-h chromium release assay, similar LAK cells caused a 73% specific chromium release at an effector: target cell ratio of 100:1; cytotoxicity was less pronounced at ratio of 6.2:1, with only 28% of the cells being lysed within the assay period (Fig. 3b). Incubation with control splenic lymphocytes resulted in no significant cytolysis (less than 15% specific chromium release).

Discussion

Effective anti-graft cell cytotoxic activity was detected in immune cells derived by lavage of the local environment of a rejecting lung graft, but this activity was not observed in peripheral immune cells. This result indicates the great potential of bronchoalveolar lavage, which allows direct

sampling of the active immune cells from within rejecting tissue. However, it was not possible to use these cytotoxicity assays to differentiate between infection and rejection, as LDMC from the infected, non-transplanted lung were also cytotoxic for donor pulmonary cells. It is possible that donor-specific lymphocytes activated during the process of rejection elsewhere in the body were recruited non-specifically to the inflamed site of the infection and were recovered by the lavage technique.

Activated porcine immune cells were capable of impairing the barrier function of cultured pulmonary cells (Fig. 3a); this process reflects dysfunction of the tight junctions between the cells. Unstimulated mononuclear immune cells had no effect on the barrier function of pulmonary cells. These results indicate that in vitro assays of epithelial tissue function may be more relevant to the analysis of the pulmonary rejection process, since the measurement of cytotoxic activity by the standard chromium release assays simply reflects the lysis of individual cells.

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References

1. Higenbottam T, Stewart S, Penketh A, Wallwork J (1988) Transbronchial lung biopsy for the diagnosis of rejection in heart-lung transplant patients. Transplantation 46: 532–539
2. Slieman C, Groussard O, Mal H, Duchatelle J-P, Dubois F, Darne C, Baldeyrou P, Jebrak G (1991) Clinical use of transbronchial biopsy in single lung transplantation. Transplantation 51: 927–928
3. Kirby JA, Wood A, Reader JA, Isted K, Hynd J, Hawkes D, Hudson L, Pepper JR (1985) Origin and immunological hyporeactivity of canine alveolar lymphocytes. Immunology 55: 531–537
4. Kirby JA, Pepper JR, Reader JA, Corbishley CM, Hudson L (1986) Precursor frequency of donor-specific lymphocytes recovered from canine lung transplants. Clin Exp Immunol 63: 334–342
5. Kirby JA, Parfett GJ, Reader JA, Pepper JR (1988) Lung transplantation in the rat: a model for study of the cellular mechanisms of allograft rejection. Immunology 63: 369–372
6. Rochat T, Casale J, Hunninghake GW, Peterson MW (1988) Neutrophil cathepsin G increases permeability of cultured type II pneumocytes. Am J Physiol 255: C603–C611
7. Cheek JM, Kim K-J, Crandall ED (1989) Tight monolayers of rat alveolar epithelial cells: bioelectric properties and active sodium transport. Am J Physiol 256: C688–C693
8. Kirby JA, Morgan JC, Shenton BK, Lennard TWJ, Proud G, Taylor RMR (1991) Renal allograft rejection: functional impairment of kidney epithelial cell monolayers mediated by lymphokine-activated killer cells and by complement. Transplantation 51: 891–895
9. Boyum A (1968) A one-stage procedure for the isolation of granulocytes and lymphocytes from human blood: general sedimentation properties of white blood cells in a 1 g gravity field. Scand J Clin Lab Invest 21 [Suppl 97]: 77
10. Brunner KT, Mauel J, Cerrotini J-C, Chapuis B (1968) Quantitative assay on immune lymphoid cells on 51-chromium labelled allogeneic targets in vitro: inhibition by isoantibody and by drugs. Immunology 14: 181–187