# PLATING INHIBITION ASSAY (PIA): A NEW TEST FOR CELL MEDIATED CYTOTOXICITY

K. Ulrichs, K.-M. Kuhlencordt and W. Müller-Ruchholtz

Department of Immunology, Institute of Hygiene and Microbiology, University of Kiel, Kiel, West Germany

The microcytotoxicity assay (MCA) of Takasugi and Klein, well established for testing cell-mediated immunity in the mouse, is however much less effective in the rat, showing far more unspecified cytolysis and less sensitivity. We therefore developed a new test system, the plasting inhibition assay (PIA). In this in-vitro test, T effector lymphocytes, increasingly with their strength of sensitization, prevent the plating of suspended target fibroblasts, i.e. their ability to adhere to surfaces such as the bottom of Terasaki microtest plates.

The essential procedures of this method, as compared with the MCA, are described in Figure 1. Results are documented in Figures 2 and 3, comparing the two test systems and using both, rat and mouse cell systems: In terms of E/T PIA is shown to be about 125 times more effective than the MCA in the rat cell system while only about 25 times in the mouse cell system. Furthermore, PIA does not appear to be restricted by unspecific cytolysis. The effector lymphocytes were identified as T cells. The specificity of target cell kill was examined against a third party strain. After 3 h incubation in the hanging drop, target cell fibroblasts, though functionally inactivated, were still viable (as shown by trypan blue exclusion). It may be mentioned that also tests for specific blocking serum factors can be performed.

Conclusions: (1) Inhibition of fibroblast adherence to surfaces is a much more sensitive and precise test to detect sensitized T lymphocytes than reduction of adhering fibroblast target cells as e.g. in the MCA. (2) This principle appears to be effective with mouse and still more with rat cell systems.

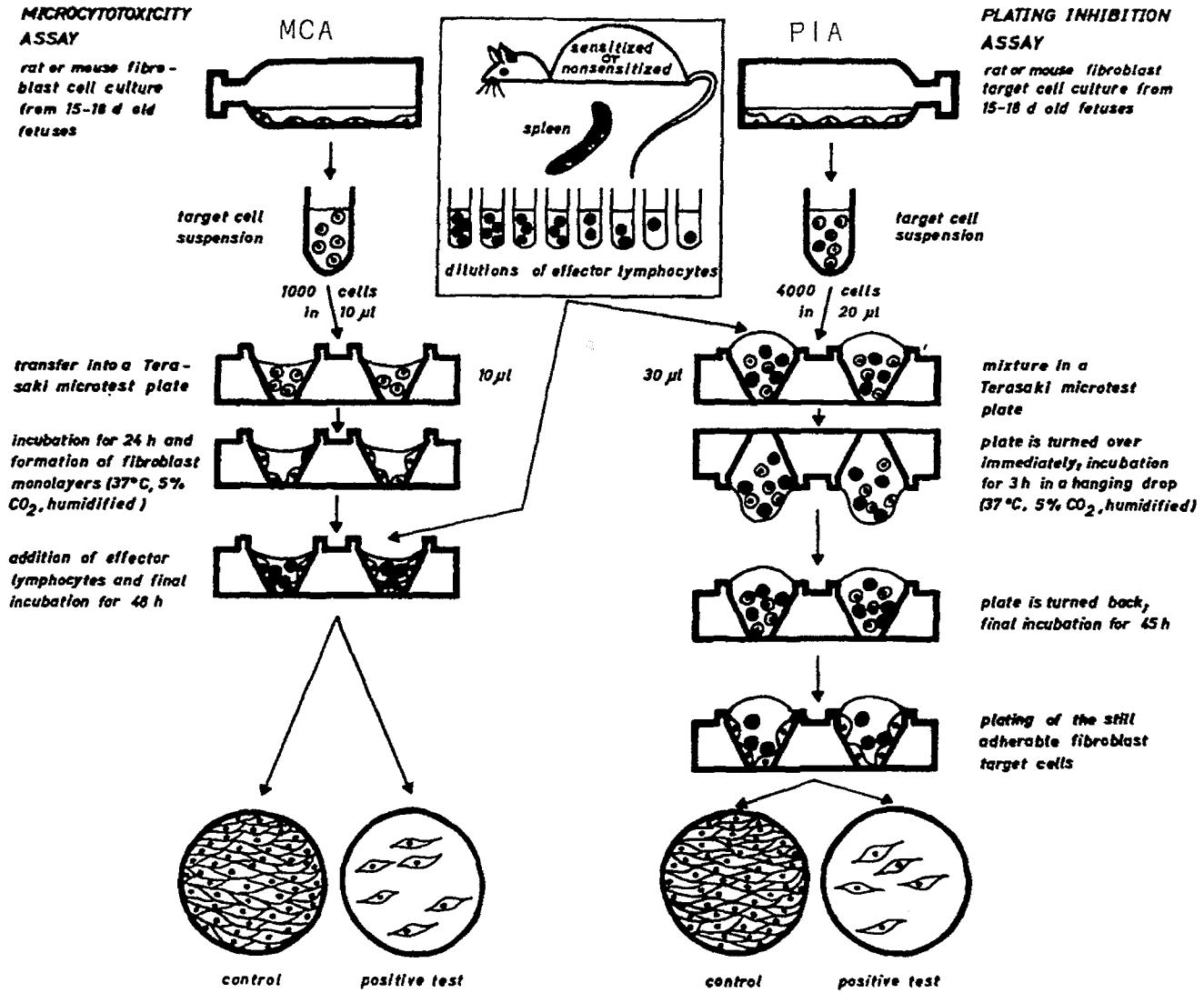


FIG. 1: Methodical procedure of PIA compared with the MCA.

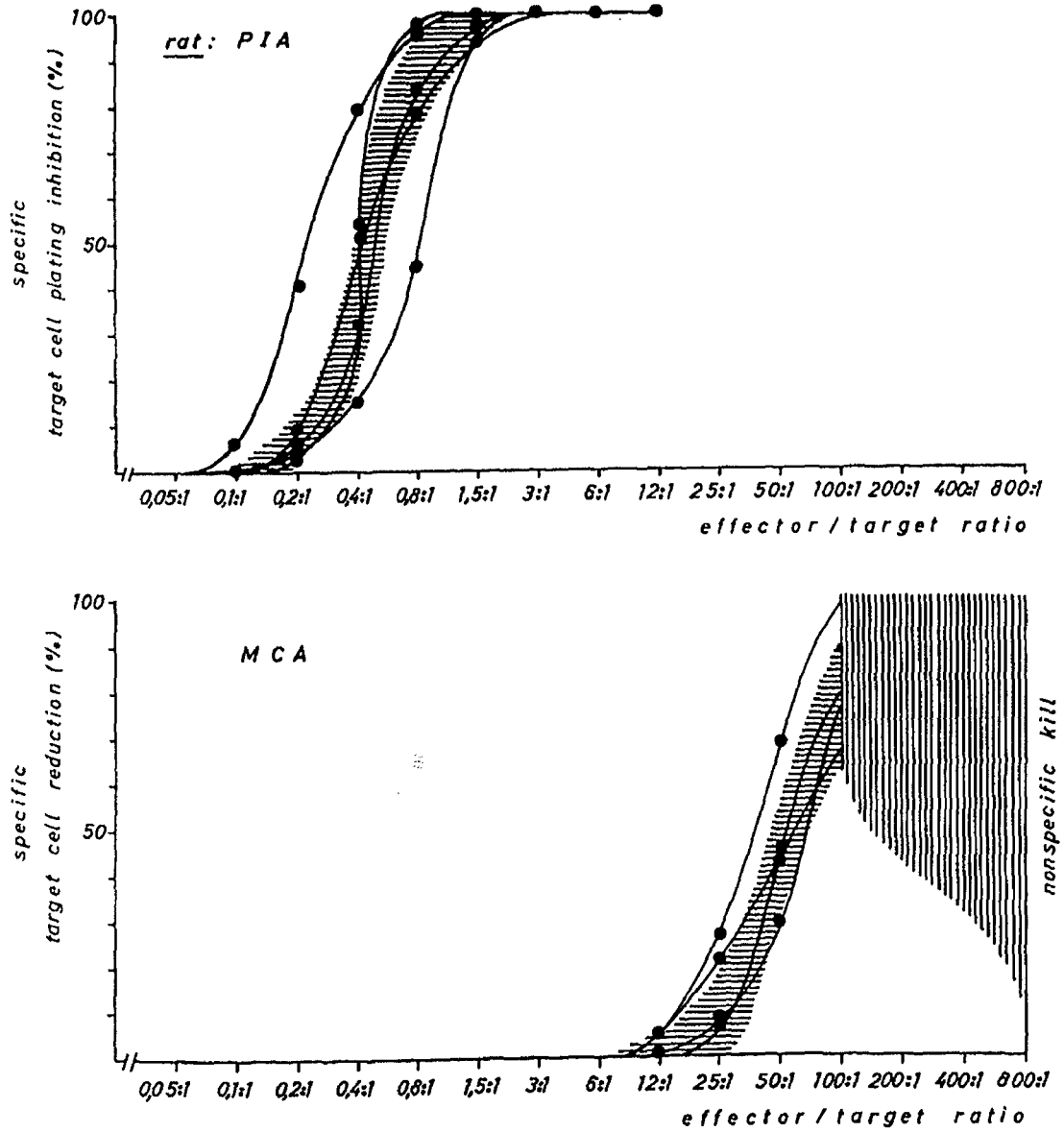


FIG. 2: Comparison of PIA with MCA in the rat sensitization: $10^8$ CAP(RT $1^c$) spleen cells i.p. into LEW (RT $1^c$), 8 d before lymphocyte preparation; target cells: CAP fibroblasts; no. of exp.: PIA: 27 tests with lymphocytes of indiv. rats in 5 groups; MCA: 27 tests with lymphocytes of indiv. rats in 4 groups; the curves represent the means of the groups; the striped area indicates the representative range of variability of one group.

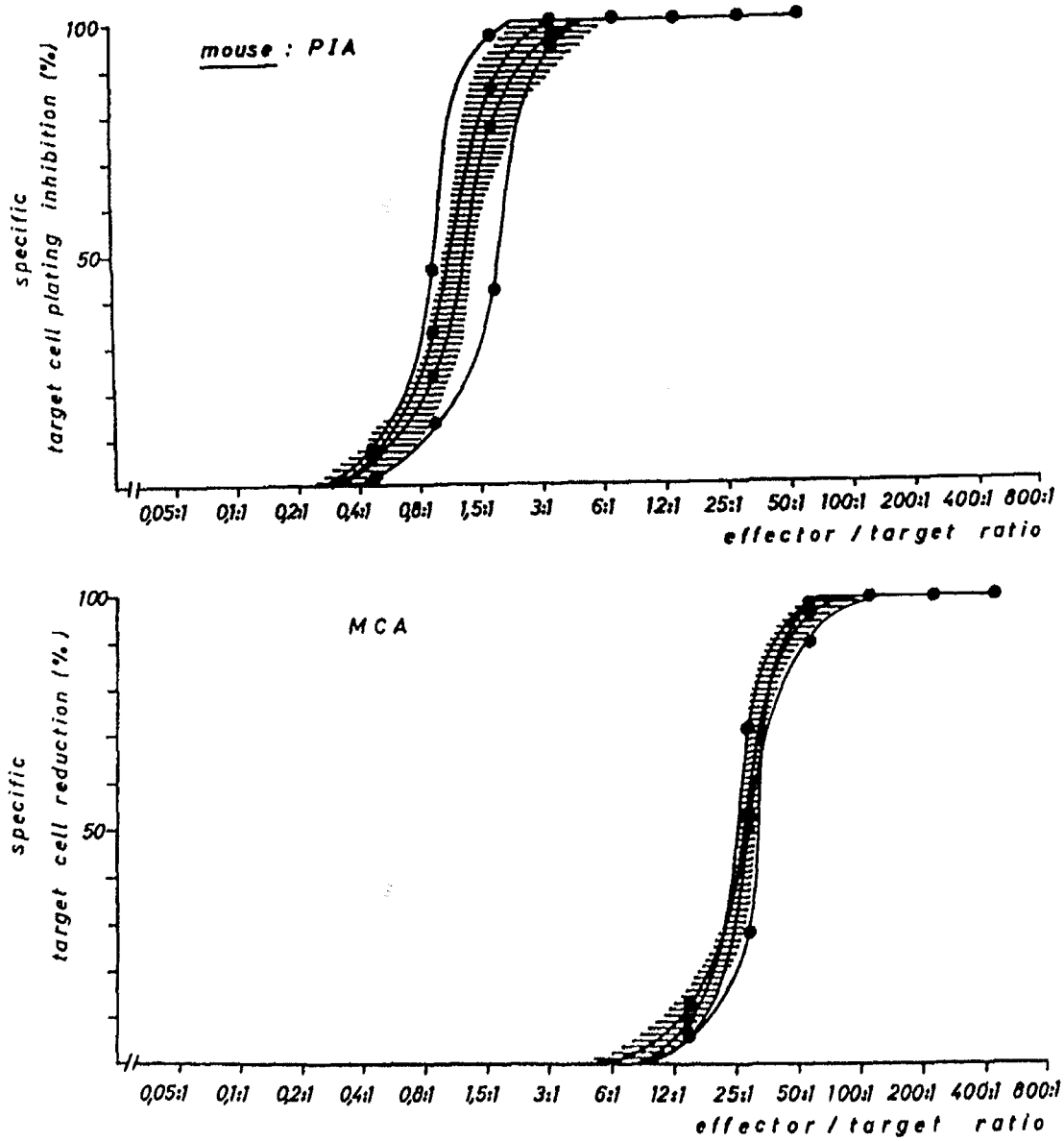


FIG. 3: Comparison of PIA with MCA in the mouse sensitization: $5 \times 10^7$ C3H(H-$2^k$) spleen cells i.p. into C57(H-$2^b$), 8 d before lymphocyte preparation; target cells: C3H fibroblasts; no. of exp.: PIA: 21 tests with lymphocytes of indiv. mice in 4 groups; MCA: 23 tests with lymphocytes of indiv. mice in 4 groups; the curves represent the means of the groups; the striped area indicates the representative range of variability of one group.