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# Characteristics of a New Anti-Rheumatic Therapy: Granulocytapheresis with G-1 Column in Humans and Rats

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Recently, it has been reported that G-1 column, a granulocytapheresis system developed by Japan Immunoresearch Laboratories Co., Ltd. (JIMRO), has significant therapeutic efficacy on 63 patients suffering from rheumatoid arthritis.[1] This study was undertaken to elucidate the characteristics of leukocyte adsorption onto G-1 column and its anti-arthritic effect in an arthritic animal model.

*Methods*: We constructed a small G-1 module containing 1 g of surface-treated cellulose acetate beads with less than 0.7 ml of dead space. About 10 ml of peripheral blood supplemented with 5 U/ml heparin-Na was taken from a normal volunteer and passed through the G-1 column for one hour. Specimens of blood were sequentially collected from the blood reservoir and the effluent pass of the G-1 circuit. Adjuvant arthritis was induced in female Lewis rats by i.d. inoculation into the hind paw with 0.05 mg of *M.butyricum*(Difco.) suspended in 0.05 ml of liquid paraffin.[2] Arthritic swelling was plethysmometrically monitored at the hind paw. Development of crippling behavior was also recorded. Arthritic rats were anesthetized with pentobarbital-Na and cannulated in the jugular veins. After i.v. injection of 500 U/kg heparin-Na, venous blood was introduced to the G-1 column. Perfusion was done in the same manner. Counts of blood leukocytes as well as harvested cells from the used column were measured by a micro-cell counter. The leukocyte population and surface molecules were analyzed by a flow cytometer with appropriate monoclonal antibodies.

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*Results and Discussions*: G-1 mainly adsorbed granulocytes from perfused human blood. Fig. 1 shows a typical result in the kinetic study of human blood cell counts in the reservoir and effluent of the G-1 circuit. The number of granulocytes in effluents were continuously lower than that in reservoir blood. A similar adsorption pattern was seen in the number of monocytes, while the number of lymphocytes did not change during the perfusion procedure. Platelets were also adsorbed very much, but only in the early phase.

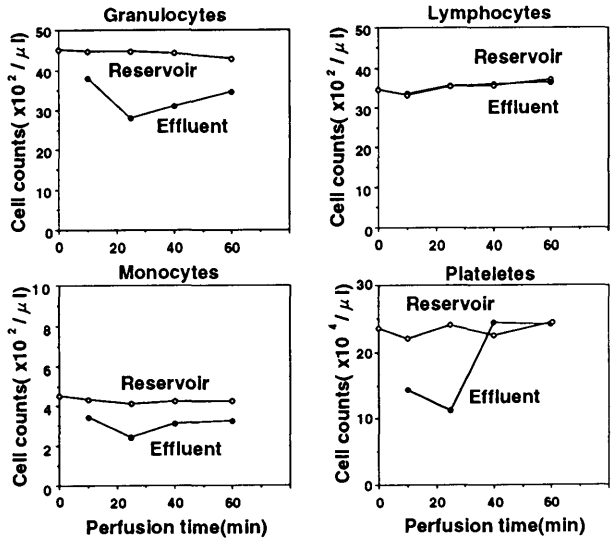


**Fig. 1 Adsorption kinetics of human blood cells during G-1 perfusion.**

The adsorbed cells harvested from G-1 beads after perfusion contained granulocytes (77%), monocytes(7%) and lymphocytes (16%). The adsorption efficiency of granulocytes was 17.9%, whereas that of T-lymphocytes was just 1.6%. These results show that G-1 adsorbed mostly granulocytes and monocytes without affecting T-lymphocyte counts.

The expressions of some adhesion molecules on granulocytes are modulated to those of activation states.[3] The cell surface density of CD62L(LECAM-1) or of CD11b(Mac-1) on granulocytes in the effluent was lower or higher than that in the reservoir,respectively. In effluent blood, higher levels of C3a and C5a, the fragments of complements in activation, were detected, but the hemolytic titer was not changed.

In arthritic rats, G-1 efficiently adsorbed the granulocytes and enhanced the expression of CD11b on the effluent granulocytes, in accordance with human blood data. These changes were abrogated by the depletion of plasma complements, using an i.v. injection of cobra venom factor 24 hours before G-1 application(Fig. 2). Complements seem to have a crucial role in the adsorption and the activation of leukocytes on G-1 beads.

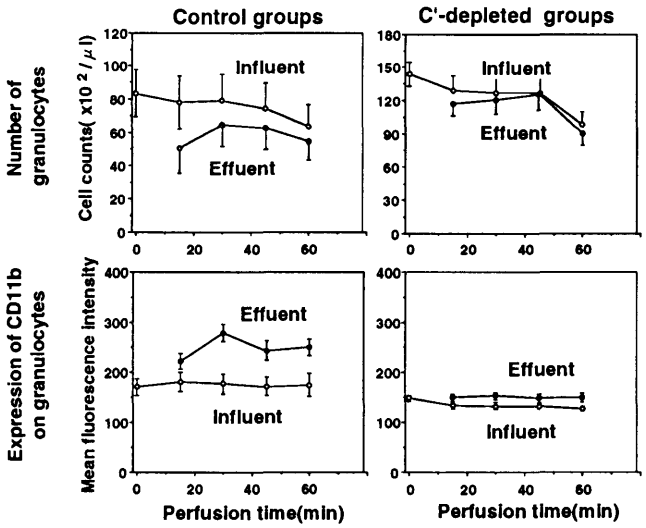


**Fig. 2 Effects of complement depletion on granulocyte adsorption and activation by G-1 in arthritic rats. (n=4, Mean±S.E.)**

In adjuvant arthritic rats, treatment by G-1 apheresis on days 7, 10 and 13 after inoculation seemed to be the best time schedule for the amelioration of clinical symptoms, such as arthritic swelling(Fig. 3) and development of crippling behavior. Histopathological analysis on day 35 revealed a distinct acceleration in the healing process of the joint inflammation.

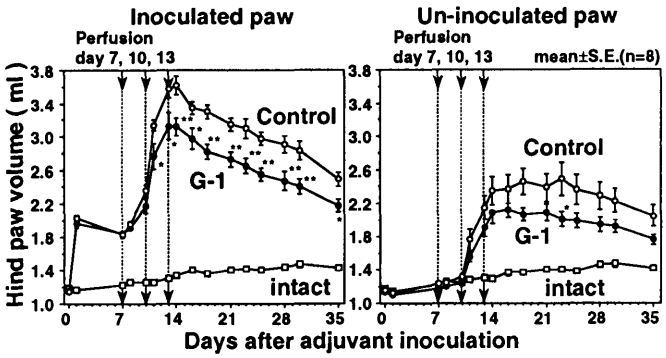


**Fig. 3 Amelioration of adjuvant arthritis by G-1. *p<0.05, **p<0.01, Dunnett test vs control.**

*Conclusion*: It was demonstrated that the G-1 column adsorbs inflammatory cells, but not T-lymphocytes, and suppresses development of arthritic symptoms in animal model.

*References*

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