——RAPID COMMUNICATION——

Jpn. J. Cancer Res. (Gann)
79, 805–808 ; July, 1988

EXPRESSION OF CR2 (C3d RECEPTOR) ON THE CELL MEMBRANES OF ADULT T CELL LEUKEMIA

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MT-2 cells, which produce human T-cell lymphotropic virus type I (HTLV-I), are known to have a complement receptor. We have established that the complement receptor is CR2 which binds C3d on immune complexes but not CR1. CR2 was also detected on ATL-3I cells but no complement receptor was detected on ATL-1K cells which lack ATL antigen (ATLA). Since CR2 is not detectable on normal T lymphocytes, the presence of CR2 on some ATL cells might suggest that ATL cells were derived from a particular minor lineage of T cells, or HTLV-I has a capacity to induce CR2, which has been demonstrated to be an α-type growth factor for B lymphocytes and to be a receptor for Epstein-Barr virus.

Key words: Complement receptor — CR2 — Adult T cell leukemia — ATL antigen

Adult T cell leukemia (ATL)[1] has been demonstrated to develop among those who have been infected with ATL virus,[2,3] which is currently called human T cell lymphotropic virus type I (HTLV-I),[4,5] mostly by vertical transmission. Although the Southern blotting analysis with cDNA of HTLV-I on lymphocytes from a healthy HTLV-I carrier revealed the incorporation of HTLV-I at heterogeneous sites,[6] the leukemia cells from a particular ATL patient showed a single band on Southern blotting analysis.[7] Therefore, ATL cells develop from a single clone among the HTLV-I-infected T lymphocytes. In this connection, it is noteworthy that a cultured cell line named MT-2 has been demonstrated to have complement receptor,[8] which is normally undetectable on T lymphocytes. Therefore we investigated the nature of the complement receptor(s) on ATL cells to establish the particular type of the receptor, since at least three distinct C3 receptors are known.[9–11] Determination of the phenotypic characteristics of ATL cells may open an approach to understanding the mechanism of the leukemic expansion of a particular lymphocyte with the HTLV-I genome.

The ATL cell lines used were MT-2,[8] ATL-3I and ATL-1K.[12] As a CR2-positive control, Raji cells, a cultured cell line of Burkitt's lymphoma, were also used. As a CR1-positive, but CR2-negative control, fresh human erythrocytes[9] of blood type O and Rh^+ were used. Cultured cell lines were grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS-RPMI). Those cells were washed with a gelatin-veronal-buffered saline (GVB), which contained 0.2% gelatin and 0.148*M* NaCl, and suspended at 5×10^6/ml in GVB. To this cell suspension was added an equal volume of 1×10^8/ml of sheep erythrocytes (E), E reacted with rabbit IgM antibody (EA), EAC4b (EA reacted with human C4), EAC4b3b (EA reacted with human C4 and C3), or EAC4d3d (prepared by incubation of EA in AKR mouse serum according to the method of Tachibana *et al.*[13]) in EDTA-GVB (a mixture of 9 volumes of GVB and 1 volume of 0.1*M* ethylenediaminetetraacetate). After incubation at 37° for 1 hr or more, rosette formation of the indicator erythrocytes on test cells was evaluated. Test cells carrying 3 or more indicator erythrocytes were regarded as positive cells.

As shown in Table I, MT-2 and ATL-3I cells formed rosettes with EAC4d3d while

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Table I. Rosette Formation on Test Cells of Sheep Erythrocytes Sensitized with IgM Antibody and Mouse Complement

Cells tested	% of cells forming rosette with indicator erythrocytes[a]			
	EA(M)C4b3b	EA(M)C4d3d	EA(G)	EA(M)
MT-2	0.6	78.8	0	0
ATL-3I	0.4	15.3	0	0
ATL-1K	0	0.5	0	0
Raji	0.6	76.2	0	0
HuE	41.5	0	0	0
PBL	30.5	29.3	17.6	0

a) EA(M) was sheep erythrocytes (E) sensitized with rabbit antibody (A) of IgM isotype. EA(G) was E sensitized with A of IgG isotype. To prepare EA(M)C4b3b, EA(M) was further reacted sequentially with guinea pig C1, human C4, guinea pig C2 and human C3 resulting in EAC1, 4b, 2a, 3b, which was then converted to EA(M)C4b3b by removing C1 and by decay dissociation of C2a through incubation in EDTA-GVB at 37° for 2 hr. EA(M)-C4d3d was prepared by the method of Tachibana *et al.*[13] Briefly, 1×10^9/ml of EA(M) were incubated with an equal volume of AKR mouse serum diluted 1:10 in GVB^{2+} at 37° for 20 min, then 2 volumes of EDTA-GVB were added and incubation was continued for 2 hr. The absence of remaining C3b molecules on the intermediate cells was verified by the lack of immune adherence reaction to HuE which have C3b receptor (CR1) but not C3d receptor (CR2).[9]

Table II. Cells Stained by Immunofluorescence with Monoclonal Antibodies[a]

Cells	Anti-CR1 (DakoC3bR)	Anti-CR2 (B2)	Anti-CR3 (OKM-1)	Anti-CD4 (OKT-4)
MT-2	0 (%)	100 (%)	0 (%)	100 (%)
ATL-3I	0	18	6	100
ATL-1K	0	0	0	100
Molt 4F	0	0	0	67
Raji	22	80	5	0
Daudi	10	75	0	0

a) Test cells were stained with FITC-labeled anti-mouse Ig following treatment with monoclonal antibodies specific to CR1, CR2, CR3 and CD4 in the presence of 3 mg/ml of normal sheep IgG.

they did not form rosettes with EA, EAC4b or EAC4b3b. On the other hand, little rosette formation with EAC4d3d was noted on ATL-1K cells, which have been reported to have no ATL antigen (ATLA) on the cell membranes.[12] In this connection, MT-1 cells, most of which were ATLA negative,[2] had no detectable complement receptor.[14] These results indicated that there might be some relationship between CR2 expression on ATL cells and that of ATLA on the leukemia cells. It seems noteworthy that CR2 has been demonstrated to be a receptor for Epstein-Barr virus (EBV).[15] Furthermore, it has also been demonstrated to function as an α-type receptor for growth factor on B lymphocytes.[16]

Although EA, EAC4b and EAC4b3b did not form rosettes with the ATL cells, we had to consider the possibility that the rosette formation of EAC4d3d on the ATLA-positive ATL cells might have some relation to the native receptor of T cells for sheep erythrocytes. To avoid direct interaction with sheep erythrocytes, which most native T cells have, we prepared complement intermediate cells with ox erythrocytes (E^{ox}), anti-E^{ox} rabbit antibody of IgM isotype, and mouse comple-

ment. Thus prepared E^{ox}AC4d3d also formed rosettes with MT-2 and ATL-3I cells. Furthermore, the presence of CR2 on MT-2 cells and ATL-3I cells was confirmed by indirect immunofluorescence staining with FITC-labeled anti-mouse Ig following treatment with anti-CR2 monoclonal antibody (Table II).

To test whether the CR2 expression might be native to ATL cells or acquired through the course of *in vitro* cultivation, fresh leukemia cells of 4 ATL patients (kindly supplied by Prof. M. Kikuchi in our school) were tested for reactivity with EAC4d3d. Before the determination, each leukemia cell preparation was passed through a nylon wool column to remove B cells and macrophages which have CR2. About 10% of the leukemia cells of 2 patients formed rosettes with EAC4d3d while little rosette formation was observed with the cells from the other 2 patients. The relationship between the CR2 expression on ATL cells and clinical manifestation of the disease is an urgent problem, since CR2 might be playing a role in the unlimited growth of the ATL cells as a kind of receptor for α-type growth factor.[16]

We are grateful to Prof. H. Hoshino for his kind provision of ATL-3I and ATL-1K cell lines, and for his valuable advice. We also thank Prof. M. Kikuchi for his kind supply of freshly frozen ATL cells. This work was supported in part by the Princess Takamatsu Cancer Research Fund and by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare.

(Received March 17, 1988/Accepted May 23, 1988)

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