A Modified Cytochemical Method for DAP IV Demonstration which Enables Simultaneous Visualization of Cell Surface Immunostaining

M.	Marzia	Cozzi
A.	Annunziata	Gloghini
A.	Antonino	Carbone

Division of Pathology, Centro di Riferimento Oncologico, Aviano, Italia

Dr. Antonino Carbone, Division of Pathology, Centro di Riferimento Oncologico, Via Pedemontana Occidentale, I-33081 Aviano (Italy)

The cytochemical demonstration of dipeptidyl amino peptidase IV (DAP IV) in human peripheral blood lymphocytes was first described by Lojda [1]. Subsequent reports have shown that this 'exopeptidase' has an apparent restriction to a particular T-cell subset, in that most T-lymphocytes bearing receptors for IgM (T_H cells) display DAP IV reactivity [2]. Crockard et al. [3], by using a combined monoclonal antibody/immunocolloidal gold technique and cytochemical method for DAP IV reactivity, demonstrated that single or several granules of DAP IV reaction products were present in 72% of OKT3+ and OKT4+ cells, whereas a significantly lower percentage of OKT8+ cells displayed positivity. Recently, a modified cytochemical method was used to show DAP IV in peripheral blood buffy coat preparations and bone marrow smears in both normal and malignant hematologic cells [4]. More recently, a modified histo-chemical method was used to show the presence of DAP IV in fixed, freeze-dried, cryostat sections of reactive and neoplastic lymphoid tissues [5]. The value of this method in the cytological classification of lymphomas was emphasized [5].

We would like to report technical results of a work undertaken in our laboratories in order to ascertain the optimal conditions for showing cytochemically the presence of DAP IV in lymphocyte subsets defined simultaneously by monoclonal antibodies in cytopspin preparations from cell suspensions.

Tonsillar and lymphoid tissues were minced and passed through a metal mesh (200–400 μ m diameter). The cells were resuspended in RPMI 1640 (Flow Lab. Inc.) with the addition of fetal calf serum at 5% (Flow Lab. Inc.); then they were washed in the same medium.

This work was supported in part by a grant from the Associazione Italiana per la Ricerca sul Cancro, Milan, Italy.

Mononuclear cells were isolated from heparinized blood samples from healthy donors on Ficoll-Isopaque (Pharmacia) density gradient. From blood, tonsils, and lymph nodes, cytopspin slides were prepared. The cytochemical demonstration of DAP IV was performed according to the method of Lojda et al. [6] with slight modifications (table I); these changes permitted good simultaneous visualization of the final reaction products of an immunocytochemical method combined with enzymocytochemistry. The method for demonstrating DAP IV activity was performed as follows: the slides were fixed 5 min in acetone; 0.4 mg/ml glycyl-prolyl-4-

methoxy- β -naphthyl-amide was dissolved in 0.5 ml of acetone and added to a solution of Fast Garnet GBC salt in 10 ml of 0.1 mol/l phosphate buffer, pH 7.2. The preparations were incubated for 17 min at room temperature; after washing, an immunocytochemical method – the APAAP technique [7] – was performed by using a panel of mouse monoclonal antibodies, including OKT4 (CD4), OKT8 (CD8), Leu-4 (CD3), Leu-14 (CD22). The enzyme-active sites of the cytoplasm showed red-brown granules; immunoreactivity for the tested antigens resulted in a blue continuous cell membrane positivity. There was no interference of the cytochemical with immunological end products; moreover, both reaction results were simultaneously evaluable. In regard to the modifications made by us of the original cytochemical method [6] (table I) it is noteworthy that acetone proved to be an adequate fixative for both enzymatic and immunological staining; it was more effective than formaline vapor for preserving the surface antigens and the enzyme reactivity. Formaline vapor was not satisfactory because of poor cell morphology and significant loss of DAP IV activity and immunoreactivity. Moreover, a yellow-

226

Cozzi/Giorgini/Carbone

Table I. Comparison of histochemical methods for showing DAP IV

Lojda et al. [6]

Khalaf et al. [5]

Present study

Tissue

Pretreatment

Substrate concentration in the incubation medium Solvent

Coupling agent Buffer

Incubation

Post-treatment 4% formaldehyde Counterstain

Rat kidney and intestine

FDCC, CIA, CA, UC, FC, FP, FV

0.5/1 mg/ml

N, N'-dimethylformamide

FBB

Phosphate or cacodylate,

pH 7.2–7.5

5–90 min at room

temperature or 37 °C

Several hours

Human tonsil and lymph node FDA

0.8 mg/ml

Dimethylformamide

FBB

Phosphate, pH 7.2

45 min at room temperature

Carazzi's haematoxylin

Human tonsil, lymph node,

peripheral blood

A

0.4 mg/ml

Acetone

FG

Phosphate, pH 7.2

17 min at room temperature

Methyl green

FDCC = Freeze-dried cryostat section coated with celloidine; FC = cryostat section after block fixation in formaldehyde; FDA = freeze-dried cryostat section fixed in acetone for 10 min; CIA = chloroform-acetone (1:1 by vol) for 2–5 min at 4°C; CA = cryostat section fixed in acetone for 5–10 min at 4°C; UC = unfixed cryostat; FP = paraffin section after formaldehyde fixation; A = acetone for 5 min at room temperature; FV = formaldehyde vapor for 2 min; FBB = Fast Blue B salt; FG = Fast Garnet GBC salt.

ish background was observed using N, N'-dimethylformamide [4,6]; on the contrary, no background occurred by using acetone as solvent. Fast Garnet GBC salt allowed to show the DAP IV reaction products more intensely than Fast Blue BB salt, because of the absence of competition with the substrate used for the immunoreaction. Finally, the incubation time of 17 min at room temperature proved to be very satisfactory for our aim: the resulting intensity of enzyme reaction permitted a good visualization of cytoplasmic DAP IV even after cell surface immunostaining; besides, precipitates were not observed.

In conclusion, by these slight modifications of the original method [6], we could combine DAP IV cyto-chemical demonstration with cell surface immunostaining by the APAAP method. In this manner, the cytochemistry of DAP IV may be used in simultaneous combination with immunological markers in the cytological classification of lymphomas.

cal staining of T lymphocytes for glycyl-proline-4-methoxy-beta-naphthylamide-peptidase. *Br J Haematol* 1982;51: 227–234.

Crockard AD, MacFarlane E, Andrews C, et al: Dipeptidyl-aminopeptidase IV activity in normal and leukemic T-cell sub-populations. *Am J Clin Pathol* 1984;82:294–299.

Khalaf MR, Bevan PC, Hayhoe FGJ: Comparative cytochemical study of dipeptidylaminopeptidase (DAP) II and IV in normal and malignant haemic cells. *J Clin Pathol* 1986;39:891–896.

Khalaf MR, Aqeel NM, Hayhoe FGJ: Histochemistry of dipeptidylaminopeptidase (DAP) II and IV in reactive lymphoid tissues and malignant lymphoma. *J Clin Pathol* 1987;40:480–485.

Lojda Z, Gossrau R, Schiebler TH: *Enzyme histochemistry: a laboratory manual*. Berlin, Springer, 1979.

Cordell JL, Falini B, Erber WB, et al.: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984;32:219–229.

References

Lojda Z: Studies on glycyl-proline naphthylamidase. I. Lymphocytes. *Histochemistry* 1977;54:299–309.

Feller AC, Heijnen CJ, Ballieux RE, et al: *Enzymehistochemi-*