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Binding of fluoresceinated lectins to normal and dinitrofluorobenzene treated human leucocytes[1]

K.R. Madyastha[2,3], P.R. Madyastha and G.W. Wood

Department of Pathology and Oncology, University of Kansas Medical Center, College of Health Sciences & Hospital, Kansas City (Kansas 66103, USA), 1 March 1978

Summary. Using fluoresceinated lectins we have shown the receptor distribution on normal human granulocytes and lymphocytes following tagging with 1-fluoro- 2, 4-dinitrobenzene (DNFB). DNP-tagged cells exhibited strong, smooth membrane staining and produced smaller patches dispersed uniformly over the entire cell surface.

We previously have reported that the agglutination of dinitrophenylated normal human peripheral blood granulocytes by concanavalin A (Con A)[4] and lymphocytes by wheat germ agglutinin (WGA)[5] was qualitatively and quantitatively similar to the reactivity of human leukemic cells with these lectins[6-8]. This observation, together with our previous finding that DNP-tagged cells were capable of evoking the production of antibodies directed against human leukemia associated antigens[9-11], suggested that dinitrophenylation may have induced surface membrane changes which normally are associated with malignancy. In a recent study[12] we have quantified the binding of Con A to DNP-tagged and untagged cells and have observed that both bound equivalent amounts of the lectin. Based on these findings we concluded that the agglutination of dinitrophenylated cells by Con A was due to a rearrangement of lectin receptors similar to that which has been reported for malignant cells[13]. In the present work, using fluoresceinated lectins, we have attempted to study the receptor redistribution on DNP-tagged granulocytes and lymphocytes following binding of Con A and WGA.

Materials and methods. Granulocytes and lymphocytes were separated from blood obtained from healthy donors by means of Ficoll-Isopaque density gradient sedimentation according to the method of Boyum[14]. Cells were tagged with 1-fluoro-2, 4 dinitrobenzene (DNFB, Sigma Chemical Co., St. Louis, Missouri) at a ratio of 10^{11} molecules of DNFB per cell as described previously[4,5]. Con A (Nutritional Biochemical Co., Cleveland, Ohio) at a concentration of 100 mg/ml and a 1% stock solution of WGA prepared[15] from wheat germ lipase, type I (Sigma Chemical Co., St. Louis, Missouri) were conjugated to fluorescein isothiocyanate (FITC) by a standard method[16]. The fluoresceinated Con A (FITC-Con A) that was employed in the present study had a final molar fluorescein to protein (F/P) ratio of 1.5 and a protein concentration of 5.5 mg/ml. The fluoresceinated WGA (FITC-WGA) had a F/P ratio of 4.5 and a protein concentration of 6.0 mg/ml. These conjugat-

Binding of FITC-Con A and FITC-WGA to untagged and DNP-tagged human granulocytes and lymphocytes[c]

FITC[a]-labeled lectin	Concentration µg/ml	Intensity of membrane fluorescence[b] Untagged granulocytes	DNP-tagged granulocytes	Untagged lymphocytes	DNP-tagged lymphocytes
Con A	128	3+	2+	3+	2+
	32	1+	1+	2+	1+
	8	1+	1+	1+	1+
	2	0	0	0	0
WGA	128	2+	2+	2+	2+
	64	1+	1+	1+	1+
	32	0	0	1+	1+
	16	0	0	0	0

[a]FITC - Fluorescein isothiocyanate; [b]The intensity of membrane staining was scored on a scale of 0-4+; [c]The determinations were done by 2 independent investigators and the findings were in good agreement.

ed lectins did retain their original agglutinability. Binding studies were performed by mixing 0.1 ml of various concentrations of FITC conjugated lectin (table) with an equal volume of cells ($7-8\times10^5$), incubating the mixture for 20 min at ambient temperature (22–24 °C), washing the cells twice with calcium and magnesium free phosphate-buffered saline (CMF-PBS) and mounting them under cover slips with a 1:1 mixture of glycerol and CMF-PBS. Inhibition studies were performed in essentially the same way except that 0.1–0.001 M (0.1 ml) of either α-methylglucopyranoside (α-MG) or N-acetylglucosamine (GlNAc), the respective sugar substrates of Con A and WGA, was added to the reaction mixture.

Results and discussion. The data in the table indicate the quantitative binding of fluoresceinated lectins. Although membrane fluorescence could not be easily quantified and did not provide a complete picture of the binding of the agglutination to normal and DNP-tagged cells, nevertheless, these data indicate that there were no significant quantitative differences in the binding of lectins even at the lowest concentrations used. In contrast to the quantitative similarity in binding, distinct qualitative differences were observed. Untagged cells that had been exposed to either lectin demonstrated focally aggregated fluorescent staining (figure, a). The aggregates that formed were irregular, varied in size and were localized in large patches. The reaction was similar to the phenomenon of capping in that movement of Con A and WGA receptors occurred. In contrast to capping, however, it was very irregular with little evidence of smooth movement of membrane components toward 1 pole of the cell. Over 90% of untagged cells demonstrated this irregular pattern of staining at all concentrations (128–256 μg/ml) suggesting that not all receptors were involved in the lectin-induced aggregation. In sharp contrast to the pattern that was seen with untagged cells, DNP-tagged granulocytes and lymphocytes exhibited only a very limited amount of the aggregate staining pattern at the highest lectin concentrations, and only 20–30% of the cells were positive. The majority of tagged cells exhibited strong, smooth membrane staining that produced smaller patches distributed uniformly over the entire cell surface (figure, b) or retained the homogeneous ring staining pattern, that was observed initially.

It is apparent from our own data[12], as well as those of others[17,18], that the difference in agglutinability of normal and DNP-tagged cells was not caused by a quantitative change in binding. The different patterns of binding of fluoresceinated lectins suggest that dinitrophenylation produced alterations in the membrane that might have facilitated agglutination. Both tagged and untagged cells had a uniform (homogeneous) distribution of lectin receptors at native form (before redistribution), as evidenced by the continuous ring-type of fluorescent staining which was observed initially. Following redistribution large, irregular, localized aggregates were seen with untagged cells which were consistent with a pre-capping stage that probably might have internalized if staining was carried out under ideal conditions (labeling for 30 min at 4 °C and further incubation for 15 min at 37 °C)[19]. In contrast to this, there appeared to have been a block or restriction of receptor mobility[20] on DNP-tagged cells thereby preventing the formation of aggregates and their subsequent internalization which resulted in uniform smooth patches over the entire cell surface or maintenace of the homogeneous ring staining pattern. This was further supported by our observation that DNP-tagged cells failed to form caps[21]. Our findings together with those of de Petris et al.[22], who reported that the lectin receptors on normal and transformed fibroblasts showed similar clustering even though the latter were more agglutinable, suggest that clustering and capping may not be necessary for agglutination. Clustering of receptor sites even may hinder the lectin induced agglutination of lymphoma cells[23]. These observations are also consistent with the finding that lymphocytes from patients with chronic lymphocytic leukemia and Hodgkin's disease were strongly agglutinable but had a diminished capacity to form caps when compared to those from normal individuals[7,8]. Thus, the restriction of receptor mobility and the subsequent failure to form large aggregates (or caps) and to internalize may facilitate the continuous availability of lectin receptors on the surface membrane of DNP-tagged cells or malignant cells and may account for their increased agglutinability by the lectins.

a b

A Binding of FITC-Con A to untreated and *B* DNFB-treated normal human granulocytes. Binding studies were performed by mixing 0.1 ml of various concentrations of FITC conjugated lectin (table) with an equal volume of cells ($7-8\times10^5$), incubating the mixture for 20 min at ambient temperature (22–24 °C).

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2 Present Address. Division of Medical Oncology, Medical College of Virginia, MCV Station, Box 230, Richmond (Virginia 23298, USA).
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