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ULTRASTRUCTURAL VISUALIZATION OF KLH ANTIGEN ON SELECTED MOUSE SPLEEN LYMPHOCYTES

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Summary

An ultrastructural study of antigen-binding spleen lymphocytes from primed mice, concentrated by a fluorescence-activated electronic cell separator, (FACS) visualized the antigen (KLH) on localized portions of the cell surface. The distribution of the KLH molecular aggregates is consistent with the "patching" and "capping" phenomenon seen by fluorescence microscopy.

The KLH aggregates were arranged in variable orientation to the cell surface. Some lay flat on the cell surface while others were perpendicular to the cell.

Introduction

De Petris and Raff (1) have recently shown the ultrastructural distribution of surface bound immunoglobin (Ig) on mouse lymphocytes by its binding to anti-mouse Ig antibody which had been conjugated to ferritin. Their studies have shown the location of the ferritin tag under differing experimental conditions of incubation temperature and metabolic inhibition, and confirm the capping phenomenon of fluorescent microscopists'. These ultrastructural studies have used poly-specific anti-mouse Ig antisera, a reagent that produces surface labeling on 30 to 50% of mouse spleen lymphocytes (the "B" cells).

We were interested in the surface distribution and behavior of antigen receptors which specifically bind a single antigen, Keyhole limpet hemocyanin (KLH). Fluorescent microscopic study of primed mouse spleen cells in this system showed 0.2 to 2% of cells which bind KLH. However, an improved version of the fluorescence-activated cell sorter (FACS) (2) developed at Stanford Medical School provides a means of purifying the fluorescent positive cells up to 100%. Fluorescein conjugated KLH (^{F}KLH) labelled cells selected with this machine were used to examine the ultrastructure of cells binding this antigen.

Methods

Spleen cell suspensions were prepared from adult BALB/cN mice primed 2-3 months earlier with KLH as described by Julius et al (3).

A flow diagram of the cell sorter is shown in Fig. 1.

Fluorescent cells, no fluorescent cells and an aliquot of the original cell suspension which had been treated with ^{F}KLH were processed for electron microscopy. KLH preparations were examined by negative staining.

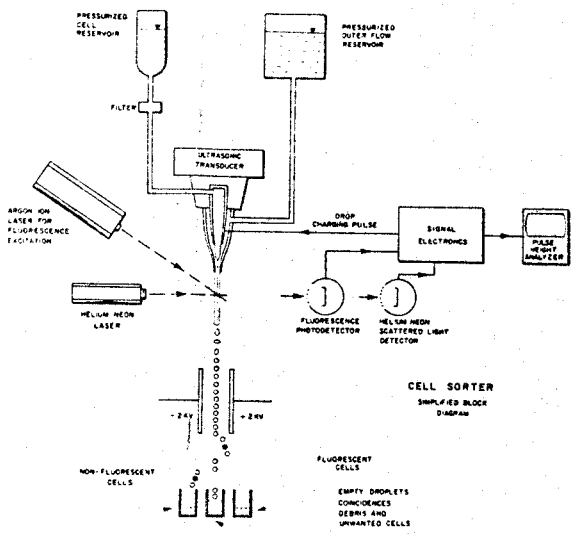


Fig. 1. Block diagram of fluorescence-activated cell sorter (FACS). The live cell suspension is introduced into the center of the fluid from the outer reservoir. The stream of cells passes the laser beams in single file and when a fluorescent cell, which conforms to preset limits intercepts the laser illumination, it will be detected. The stream is charged at the point of droplet formation. Droplets are formed by mild ultrasonic shaking at a rate of 40,000 p/sec. Charged droplets are deflected electrostatically. Reproduced from Clin.Chem. [2]

Results

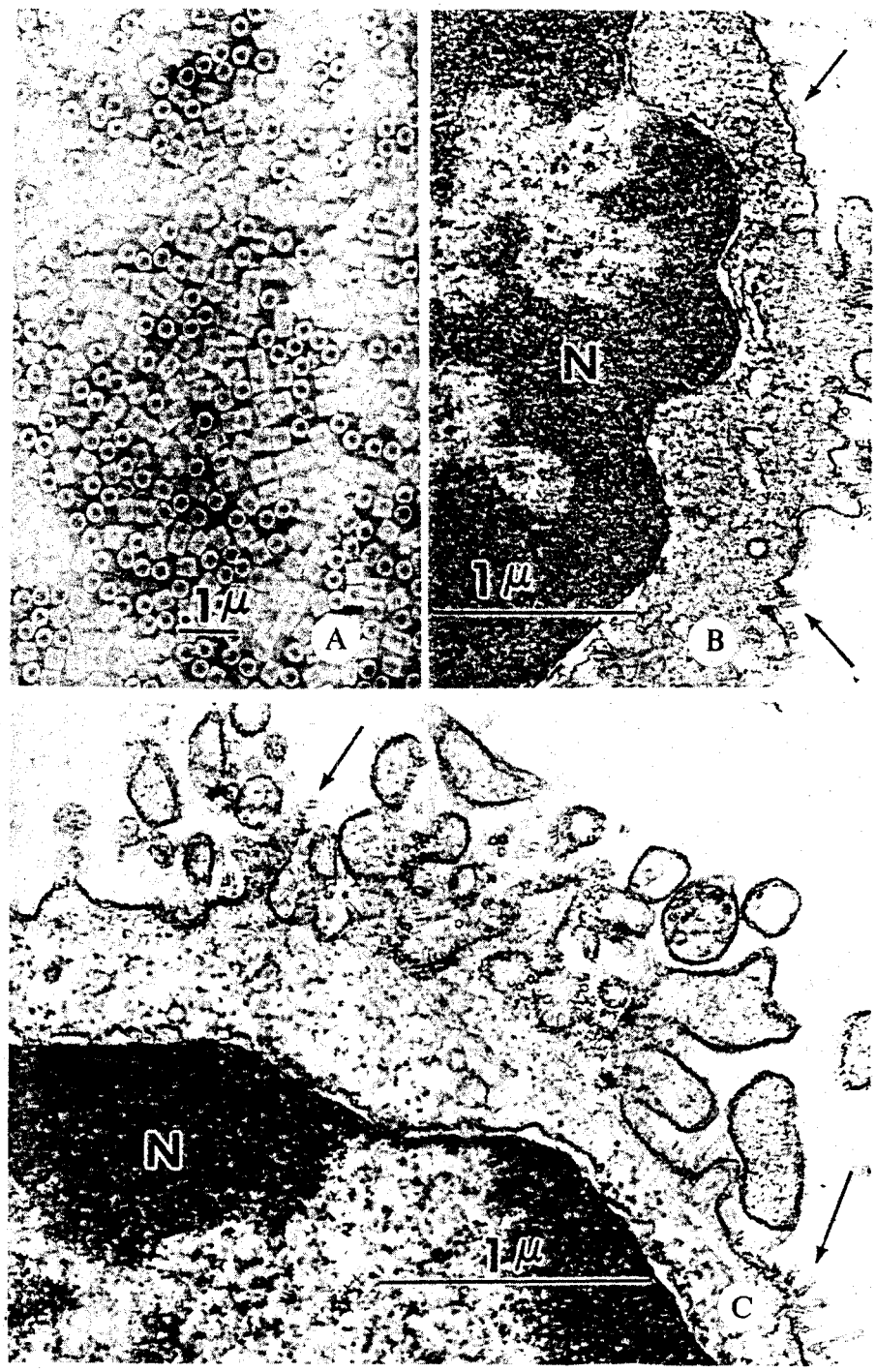
The initial cell suspension had 0.7% fluorescent cells. A selected cell population was re-run through the machine and produced a cell suspension (84% positive fluorescent) which was processed for ultrastructural studies.

The ^{F}KLH used in one experiment showed uniform molecular aggregates when negatively stained. Fig. 2a.

These aggregated molecules, 320 Å in cross section and 180-500 Å long, were produced consistently when either KLH or ^{F}KLH were treated with glutaraldehyde in a procedure by Thompson and Wofsy (4). KLH is seen on the surface of the cells in Fig. 2b and 2c.

The predominant fluorescent distribution in this preparation was a capping

Fig. 2. A - negative stained ^{F}KLH treated with glutaraldehyde. scale 0.1 micron. B and C - KLH aggregates (arrows) on a lymphocyte surface in a capped area (c) scale 1 micron.


1μ
A
N
1μ
B
N
1μ
C

pattern and these cuts are apparently thru a capped area.

No KLH molecular aggregates were seen on the surface of the cells in the non fluorescent fraction.

A morphological examination, by E.M., of the fluorescent and non fluorescent groups showed no marked difference.

Discussion

These observations demonstrate clearly the position of antigen bound to lymphocytes presumably on an antibody molecule produced by the lymphocyte. The distribution of these KLH molecules is consistent with patching and capping processes seen by fluorescent microscopy in these experiments, and also in fluorescent and ultrastructural studies of poly-specific surface Ig distribution. The varied orientation of the KLH aggregates to the surface of the lymphocytes is also consistent with the concept of the multivalent character of the KLH molecular aggregates.

References

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