EXCESS IgD BEARING LYMPHOCYTES IN PATIENTS WITH MALIGNANT MELANOMA

S. MALKA, C. J. OON AND J. R. HOBBS

From the Tumour Biology Group, Westminster Hospital, London S.W.1

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Summary.—B lymphocytes from the peripheral blood of 15 melanoma patients and 14 normal adults were studied using immunofluorescence to IgA, IgG, IgD and IgM surface markers. The total number of peripheral blood B lymphocytes was increased in the melanoma patients (1048/mm³) compared with normal controls (504) (P<0.01). The expression of excess IgD bearing lymphocytes in these melanoma patients may reflect a derepression of oncofoetal type.

High levels of IgD bearing lymphocytes are found in the foetus, newborn and children, but these fall to low levels in the peripheral blood of adults (Rowe et al., 1973).

In a routine examination of B lymphocyte function in various malignancies, we discovered that a high number of the peripheral lymphocytes in melanoma patients had IgD on their surface. To our knowledge this finding has not been reported previously.

MATERIALS AND METHODS

Patients.—Fifteen patients with histologically proven malignant melanoma from Stage I to Stage III were studied; 14 normal healthy adult laboratory staff (age 20–40 years) were used as controls.

Samples.—10–20 ml heparinized blood. Lymphocytes were separated using Lymphoprep (Nyegaard & Co., Oslo). Lymphocyte morphology, staining with specific antisera, examination for fluorescent labelling and specificity controls were carried out using the method of McLaughlin et al. (1973).

Total and differential white cell counts were done in each case.

Antisera.—Commercially obtained fluorescein conjugated monospecific antisera to human IgG, IgA, IgM, and IgD were used (Behringwerke AG, Batch Nos: 572A, F504Q, 580B and F492P respectively).

RESULTS

Morphology

More than 90% of cells obtained were lymphocytes. The remaining cells were polymorphs and monocytes.

Staining

The varieties of staining (cells heavily stained with large dots, others with numerous smaller dots, and others with ring or cap formation) as described previously, were observed throughout in both the normal controls and the melanoma patients. There was no evidence to suggest any maturation error or synchronization. Trypsinization and washing in a few studies resulted in regeneration of the same proportions of cells.

Melanoma patients

The total numbers of Ig-bearing lymphocytes in the peripheral blood were increased (P < 0.01) and there was a corresponding diminution in the number of non-Ig-bearing cells (P < 0.001), see Table which shows that this is due to an increase in the total number of lymphocytes bearing IgG (P < 0.02) or IgD, especially the latter, which were some 5 times more numerous than in controls.
TABLE.—The Percentage and Absolute Number of Ig-Bearing Lymphocytes in 14 Normal Individuals and 15 Melanoma Patients

| Group   | Lymphocytes (%) | Observed range | Log-normal calculated |
|---------|-----------------|----------------|-----------------------|
| IgG     |                 |                | Mean ±2 s.d.          |
| Normal  | 8–16            | 101–354        | 195 103–369           |
| Melanoma| 9–35            | 166–857        | 322 92–1122           |
| IgD     |                 |                | t_{13} P              |
| Normal  | 0–8             | 0–266          | 80 0–397              |
| Melanoma| 1–48            | 16–1385        | 422 18–1231           |
| IgA     |                 |                | 14–440 94 15–590      |
| Normal  | 2–12            | 14–440         | 94 15–590             |
| Melanoma| 0–20            | 0–746          | 114 6–2089            |
| IgM     |                 |                | 0–43 N.S.*            |
| Normal  | 4–13            | 58–442         | 109 40–301            |
| Melanoma| 0–17            | 0–393          | 98 5–1791             |
| Total Ig-bearing |           | 21–96          | 426–3402 1048 241–4560 |
| Total non-Ig-bearing |   | 59–85          | 723–2922 1343 676–2667 |
| Normal  | 2–79            | 47–1604        | 352 42–2974           |
| Melanoma|                |                |                      |

* N.S. = not significant.

(P < 0.01). There was no significant difference for IgA or IgM bearing cells.

The study was too small to differentiate the differences between those in "remission" and those with active disease clinically, though the patients with clinically apparent disease had consistently high peripheral IgD lymphocyte counts of greater than 270/mm³ (above the range of 0–266/mm³ in normal adults).

The Figure shows the frequency distribution observed in normal controls and melanoma patients for IgG and IgD bearing lymphocytes. In melanoma patients these are increased for IgG and IgD.
DISCUSSION

High levels of IgD bearing lymphocytes are reported in newborns, but hitherto there have been no published reports of their presence in malignant diseases in man.

In separate studies Rowe et al. (1973) have used percentages in expressing their results and have shown that mean levels of IgD bearing lymphocytes were 14.5% in cord blood of newborn and 3.8% in adults. However, percentages are not satisfactory expressions of the quantity of lymphocytes that were studied.

Using numbers/mm³ the log-normal distributions of both Ig-bearing and non-Ig-bearing lymphocytes become obvious (see Figure, McLaughlin et al. (1973)) and correct log-normal statistics can be applied to assess significance.

The decrease in non-Ig-bearing lymphocytes, especially in patients in relapse, is in agreement with the absence of the mixed lymphocyte reaction found in half of the patients with Stage II (and worse) melanomatosis (Butterworth et al., 1974). It also accords with a significant reduction in melanoma patients of peripheral lymphocytes (presumably “T”) forming spontaneous rosettes with sheep erythrocytes (Bourgoin et al., unpublished).

The finding of increased Ig-bearing lymphocytes, mainly IgD-bearing cells, may have two causes. With a fall of T lymphocyte function, more B lymphocytes might emerge and in such a situation of regeneration there might be an excess of IgD-bearing cells. We are exploring this in similar conditions. Alternatively, there may be a true foetal throwback. Recently, Carrel and Theilkaes (1973) have found an antigen in melanoma patients, and the antisera to this antigen also cross react with antigens in patients with neuroblastoma and ganglioneuroma. Since the precursor cells, common to all three tumours, are thought to originate in the embryonic neural tube, it is possible that this common antigen may be of oncofoetal origin. Our finding of an increase in IgD-bearing lymphocytes in our melanoma patients could therefore support a hypothesis of a possible oncofoetal throwback in melanomatosis. This may be derepression of an oncofoetal type.

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