SEARCH FOR AN INFLUENCE OF NATURAL IMMUNITY ON THE LUNG COLONY ASSAY OF A SYNGENEIC TRANSPLANTED MURINE TUMOUR

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The existence of natural immunity against tumour has been inferred from the observation that lymphocytes from an animal with no previous contact with a tumour may exert a cytotoxic effect on the tumour cells (Herberman et al., 1975).

Several workers have previously reported that prior whole-body irradiation (WBI) of mice increased the number of tumour nodules in the lungs of mice after their i.v. injection with a single-cell suspension of tumour cells (Withers and Milas, 1973; van den Brenk et al., 1973a; Thompson, 1974). One of us (Janik, 1976) has recently confirmed this effect, using the tumour employed in the present experiments. At least two mechanisms for the effect have been suggested: (1) radiation-induced lung damage increases the probability of tumour cells seeding in the lung (van den Brenk et al., 1973b; Thompson, 1974); and (2) WBI suppresses immunity against the tumour (Rosenau and Moon, 1967). In the experiments reported here, we have examined the second interpretation by attempting to determine which part of the immunological system may be involved. We have compared the results of lung-colony assays using normal mice, WBI mice which had been reconstituted with marrow cells (BM) or with a mixture of BM and thymus cells (T), and thymectomized mice. We studied also the effect of non-specific stimulation of immunity (Baldwin and Pim, 1973).

Mice and tumour.—The experiments used a tumour (Sarcoma L-1) which arose spontaneously in the lung of a BALB/c mouse and had been maintained in that strain; material from 9th–13th serial passages was used. Recipient mice were male or female BALB/c mice, except in one experiment in which mice of inbred strain BN/b were used to provide an allografted system. These served as control for reconstitution procedure.

Lung-colony assays.—Single-cell suspensions were prepared from enzymatically disintegrated tumour as described previously (Janik, 1976), and were injected i.v. in a volume of 0·2 ml into mice lightly anaesthetized with ether. Lung tumour nodules were counted 2 weeks after injection, using essentially the same technique as that described by Hill and Bush (1969).

Irradiation.—Mice were exposed to 900 rad WBI delivered from a 60Co γ-ray source at a rate of 108 rad/min.

Reconstitution.—WBI mice received i.v. $20 \times 10^6$ BM or $20 \times 10^6$ BM + $40 \times 10^6$ T. WBI mice receiving BM only, showed very poor production of IgG against sheep red blood cells (SRB), as indicated by haemolytic plaque assay (Table III). Tumour cells were injected 2 weeks after reconstitution.

Thymectomy.—Mice were thymectomized one day after birth and were used for assay at 8–10 weeks of age; these showed reduced production of IgG (Table III). Mice showing residual thymus at autopsy were excluded from results.
TABLE I.—Mean Numbers (± s.d.) of Tumour Nodules in Lungs of Normal Mice, or WBI Mice which were Reconstituted with either T + BM or BM only, following their i.v. Injection with $10^5$ Tumour Cells

|                | BALB/c |                | BN/b   |
|----------------|--------|----------------|--------|
|                | Normal | WBI            | BM only| WBI          |
| BALB/c T + BM  | 7.3 ± 0.5 | 6.4 ± 1.1  | 14.3 ± 2.2 | 0 |
| BALB/c BM only | 12.5 ± 2.2 | 12.5 ± 2.2 |          |          |

In parentheses, the number of mice

TABLE II.—Mean Number (± s.d.) of Tumour Nodules in Lungs of Variously Pretreated Mice which Received Tumour Cells i.v.

| No of tumour cells i.v. (× 10⁴) | Normal | Thymectomy | Normal + BCG | Thymectomy + BCG |
|---------------------------------|--------|------------|--------------|------------------|
| 12                              | 8.7 ± 1.6 | 6.5 ± 2.2 | 3.4 ± 0.7   | 7.2 ± 0.5   |
| (7)                             | (7)     | (7)        | (8)          | (9)            |
| 25                              | 17.2 ± 4.4 | 16.4 ± 3.2 | 3.4 ± 0.7   | 7.2 ± 0.5   |
| (10)                            | (8)     | (8)        | (8)          | (9)            |
| 25                              | 11.1 ± 2  | 16.4 ± 3.2 | 3.4 ± 0.7   | 7.2 ± 0.5   |
| (8)                             | (8)     | (8)        | (8)          | (9)            |

In parentheses, the number of mice

TABLE III.—Numbers of Plaque-forming Cells (PFC) per 10⁶ Spleen Cells of Thymectomy BM and BM + T Reconstituted Mice. Mean ± s.d. (numbers of mice in parenthesis) or individual values

|                | Days after immunization with SRBC | PFC/10⁶ Spleen cell |
|----------------|-----------------------------------|---------------------|
|                | Direct                            | Indirect            |
| Treatment      |                                   |                     |
| Thymectomy     | 8                                 | 10.6 ± 3.2          | 31.2 ± 2.8 |
|                | (5)                               | (5)                 |
| Normal         | 8                                 | 21.4 ± 3.0          | 100.1 ± 11.2 |
|                | (4)                               | (4)                 |
| BM reconst.    | 10                                | 15, 18              | 54, 60     |
| BM + T reconst.| 10                                | 25, 30              | 387, 340   |
| Normal         | 10                                | 33, 40              | 640, 530   |

BCG.—Doses of 0.1 mg of BCG (Biomed, Poland) were injected i.p. 10 and 2 days before i.v. injection of tumour cells.

Haemolytic direct plaques.—These were assayed by the micromethod of Cunningham and Szemberg (1968). Indirect plaques were made in an identical manner, but the medium contained 10% rabbit antismouse-IgG serum. Mice were immunized with SRBC 2 weeks after reconstitution.

Table I shows that WBI BALB/c mice which were reconstituted with BM alone, yielded a significantly higher lung nodule count than normal mice, whereas those reconstituted with BM + T yielded counts which were not significantly different from those for normal mice. This last finding would seem to exclude radiation-induced damage from having any significant influence on lung-nodule formation. The findings for allografted BN/b mice (Table I) show that thymus-derived cells are required for resistance to allografted tumour cells and, together with drastic reduction of IgG in BM-reconstituted mice, as indicated by indirect plaques (Table III) proves that reconstitution with
BM cells alone leads to diminution of T-cell function.

Table II shows that thymectomy did not increase the yield of lung nodules above that for normal mice, whereas the thymus-dependent part of the immunological response (indirect plaques) against SRBC was drastically reduced (Table III). Thus the evidence for a role of thymus-derived cells in maintaining the level of tumour receptivity in normal mice is conflicting, in that restoration of thymus cells to WBI cancels the enhancement due to WBI, whereas thymectomy does not alter receptivity.

Table II shows that prior administration of BCG significantly reduces the yield of lung nodules in both normal and thymectomized mice. It has been demonstrated previously that BCG stimulates tumour immunity (Baldwin and Pim, 1973) and that this effect does not require the presence of intact thymus (Sadler and Castro, 1976). Christie and Banford (1975), however, asserted that the stimulating effect of C. parvum requires the presence of theta-positive cells to activate macrophages.

Our overall conclusion from these studies is that T lymphocytes are not “natural” killers of tumour cells in vivo and do not mediate the stimulating effect of BCG. On the other hand, T lymphocytes are required for restoration of the natural immunity destroyed by irradiation.

We acknowledge the skilful technical assistance of Mrs K. Jagóra and Miss I. Mostowska.

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