Lymphocyte Beryllium Binding: Relationship to Development of Delayed Beryllium Hypersensitivity

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Abstract

Beryllium binding to human and both inbred and outbred guinea pig lymphocytes has been characterized by Scatchard binding analysis. In isotonic media, only one class of binding sites, presumed to be located on the outer cell surface, was identified with a relatively low affinity (K_A $3-4 \times 10^5 M^{-1}$). In hypotonic media in which the cells rupture, an additional class of binding sites, probably in the cell nucleus, is revealed with a higher affinity (K_A $1-2 \times 10^6 M^{-1}$). Preliminary investigation of the high affinity binding in strain XIII guinea pig peripheral blood lymphocytes provides evidence that a greater level of beryllium binding may be predictive of the potential to express delayed beryllium hypersensitivity.

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Delayed type hypersensitivity to beryllium (Be) compounds is a response demonstrable in both man and experimental animals [1]. Furthermore, lymphocytes derived from sensitized individuals can be shown to produce macrophage migration inhibition factor (MIF) [2-4] or undergo transformation when exposed to Be salts in vitro [5-6]. It would be useful to be able to distinguish responders from nonresponders, particularly prior to employment of man in the beryllium industry, since at present only diagnostic tests for Be disease are available and initial use of a patch test is precluded as this in itself might sensitize predisposed individuals. Little is known about the mechanisms by which Be interacts with cells of the immune system or the factors related to predisposition to Be hypersensitivity. Notwithstanding the demonstration that Be can combine with proteins of the skin [7], more recent studies have suggested that Be may interact directly with lymphocytes without prior complexing to a macromolecule [3]. We have therefore examined the binding characteristics of Be to human and guinea pig lymphocytes and in addition using a guinea pig animal model attempted to relate these parameters to subsequent development of Be contact hypersensitivity with a view to devising a predictive in vitro test.

Peripheral blood lymphocytes from non-Be exposed human volunteers and three strains of guinea

pig (outbred; Dunkin Hartley; inbred strains II and XIII) were isolated and the Be binding properties measured in vitro by Scatchard binding analysis [8]. In this treatment, when bound Be measured at various Be concentrations is plotted against the bound/free ratio, a negative slope curve is obtained from which the binding can be characterised in terms of a defined affinity constant (K_A). Figure 1 illustrates the binding curves obtained and shows that lymphocytes in isotonic media give rise to a linear Scatchard binding plot indicating the presence of one class of binding site with a K_A of $3-4 \times 10^5 M^{-1}$. Since lymphocytes remain intact under these

conditions, and it is known that Be salts do not readily penetrate cell membranes [9], it is concluded that these binding sites are located on the outer surface of the lymphocyte membranes and therefore may be involved in membrane activation processes elicited by Be compounds.

When lymphocytes were incubated in a hypotonic medium, cell rupture was seen, as evidenced by penetration of trypan blue exclusion dye. Under these conditions, more complex Scatchard Be binding plots were obtained comprising a series of approximately parallel curves for the different lymphocyte samples examined (fig. 1). It may reasonably be assumed that the plotted curves are made up of the previously determined component ($K_a 3-4 \times 10^5 M^{-1}$) and an additional class of higher affinity binding sites, the K_a values of which

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Fig. 1. Scatchard analysis of beryllium binding to peripheral blood lymphocytes. Cells (human, O; guinea pig inbred strain XI-II, •; II, ▲; outbred Dunkin-Hartley, ■) purified on Lymphoprep (Nyegaard) and washed twice with RPMI (Gibco), followed by 0.15 M saline were incubated in 10 mMTris-HCl pH 6.0 (1 ml ca. 0.1 mg cell protein; 2×10^6 cells) in the presence of 0.5, 1, 2, 5, and 10 μM radiolabelled (7Be) beryllium sulphosalicylate [10] at 30°C for 30 min. Bound Be (nmol/mg cell protein) was determined for measurement of radiolabel present in the centrifuged (2,000 g) cell pellet and free concentration (μM) measured in the supernatant. □ = Human or guinea pig lymphocytes incubated as above but in 10 mMTris-HCl pH 6.0, 0.15 M saline. Data points in each case represent mean values of 4 separate determinations.

Bound, nmol Be/mg protein

Fig. 2. Scatchard analysis of beryllium binding to guinea pig strain XIII lymph node and thymus lymphocytes. Cells ($\bullet$ = lymph node; $\circ$ = thymus) prepared as previously described [3] incubated in the presence of 1, 2, 5, 10, 15 and 30 μ M beryllium sulphosalicylate, as detailed in figure 1.

Table I. Correlation of guinea-pig strain XIII lymphocyte beryllium binding with beryllium hypersensitivity

Mean values $\pm$ SD.

1 Individual blood samples (2–4 ml) removed by cardiac puncture. Beryllium binding determined in presence of 0.5 μ M beryllium sulphosalicylate, as described in figure 1.

2 1 days after cardiac puncture animals sensitized with 2 applications to a shaved flank of 20% BeS04 in EWT (45% ethoxyethanol, 45% water, 10% 'Tween' 80) followed 1 week later with a challenge on the untreated flank with BeS04 as described previously [3]. A positive reaction was taken as a definite reddening and thickening of the skin at 24–48 h compared with EWT treatment alone.

were calculated to be $1 \times 10^6 \text{ M}^{-1}$ (human); $1 \times 10^6 \text{ M}^{-1}$ (guinea pig strain II), $1.7 \times 10^6 \text{ M}^{-1}$ (guinea pig strain XIII) and $1.5 \times 10^6 \text{ M}^{-1}$ (guinea pig Dunkin-Hartley). The similarities in the K_a values would suggest that Be binding is to the same cellular component in each case and since the cell membrane is known to be broken, could be either to a sub-cellular organelle or possibly the inner side of the lymphocyte membrane. Previous sub-cellular fractionation studies with rat liver have established that Be exhibits a selective high affinity binding to cell nuclei ($K_a = 2 \times 10^6 \text{ M}^{-1}$) [10, 11] comparable with that measured in ruptured lymphocytes and therefore nuclei are probably the subcellular site involved in the present case. Thus, it can be generally concluded that lymphocytes possess two types of Be binding sites; relatively low affinity sites residing in the cell membrane and higher affinity sites probably located in the cell nucleus. This conclusion is supported by the demonstration that lymphocytes derived from lymph nodes and thymus show comparable Be binding characteristics (fig. 2; K_a (low) $1.2 \times 10^5 \text{ M}^{-1}$; K_a (high) $1.5 \times 10^6 \text{ M}^{-1}$).

Although the high affinity binding constants were similar for human and guinea pig lymphocytes, the

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actual level (capacity) of Be binding measured was different for the various systems tested. Consequently, at low Be concentrations (0.5–1 μ M), the lymphocyte binding capacity of the

different sources decreased in the order strain XIII > Dunkin-Hartley > strain 11 = human. Guinea pig strain XIII, in our hands, also proved to be the most reproducible and responsive animal model in skin hypersensitivity tests, despite an earlier report that strain II should have been more suitable [12].

Earlier studies have shown that the nuclear components Be binds to are specifically the acidic non-histone nuclear proteins [11] which are known to regulate cell division and gene transcription [13]. Differences in the interaction of Be with this group of regulatory proteins might therefore reflect differences in the degree of responsiveness to Be and consequently the level of hypersensitivity. Accordingly, we attempted to correlate, in guinea pig strain XIII, the level of high affinity Be binding to lymphocytes with the potential for subsequent skin hypersensitivity to Be. The results (table I) show that in only 15% of the animals were we able to demonstrate delayed Be hypersensitivity, but this appeared to be correlated with higher Be binding capacity of the lymphocytes prior to sensitization, if binding was recorded with respect to cell protein but not to cell number. This suggests the presence of altered proportions of high affinity Be binding proteins and non-Be binding proteins in lymphocytes from guinea pigs potentially hypersensitive to Be. In the absence of further fractionation studies, it is not clear if the increased binding levels reflect properties of all the lymphocytes present, or indicates the presence of selective sub-sets of lymphocytes with different relative Be binding capacities, a conclusion which might be supported by the broad variation and overlap in the binding data values observed in the present investigation. However, with further refinement, the method might form the basis for a predictive test for Be hypersensitivity, particularly if it can be demonstrated more conclusively in a more responsive animal model system.

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