A Histochemical Study of Immune Destruction of the Lymphopoietic System

LANDSTEINER[1] first suggested that certain peptides and non-antigenic molecules such as glucose could be made antigenic by coupling the molecule to an antigenic protein carrier. Others have since shown that a number of non-antigenic or relatively non-antigenic substances could be made antigenic by similar means. Experiments in this laboratory have provided evidence that a state of active immunity could be established to a variety of autologous animal and human neoplastic tissue[2], normal autologous lymphoid tissue[3] and isologous and autologous serum proteins[4] by means of coupling cellular elements of these tissues or components of the serum proteins to highly antigenic proteins and then reintroducing these complexes into the host from which the tissue or serum protein was originally obtained. Furthermore, the antibodies elicited by this procedure were found to have marked specificity suggestive that an autoimmune state had been induced toward the neoplastic or lymphoid tissue or serum protein.

In the present study, immune destruction of lymphopoietic tissue was produced (Figure 1). A study of lysosomal galactosidase content of the destroyed lymphopoietic system was performed since it has been suggested that there are aberations in lysosomal enzyme content in immunopathologic states (Figure 2). This study was facilitated by the availability of a new group of histochemical substrates, substituted indolyl β-galatopyranosides, which were synthesized in our laboratory[5,6].

Bisdiazobenzidine (BDB) was prepared by the method of KABAT and MAYER[7]. Three ml of 6*N* HCl were added to 0.46 g of benzidine dissolved in 100 ml of water. This mixture was chilled in an ice-salt bath to 0 °C at which time it was diazotized by dropwise addition, with constant stirring, of 0.35 g of sodium nitrite dissolved in 5 ml of water. This mixture was allowed to react for 30 min at 0 °C after which the aliquots were removed and quick frozen in small vials at — 70 °C in a solidified carbon dioxide (dry ice)-acetone bath. The vials were then quickly transferred to a deep freeze (— 20 °C) where they were stored until needed.

Incomplete Freund's adjuvant was prepared by thoroughly blending 3 parts of aquaphor with 1 part of heavy liquid petrolatum. Phosphate buffer of molarity 0.2 and pH 7.2 was prepared by mixing 28 ml of a 0.2*M* NaH_2PO_4 with 72 ml of 0.2*M* Na_2HPO_4. The pH was checked on a Beckman Expanded Scale pH meter and adjusted when necessary. Lymphoid cell suspensions were prepared from a lymph node which was removed from the abdominal cavity of a Fischer rat. This lymph node was divided into several pieces, frozen, and used for the entire duration of immunization. A small portion of this node was pressed through a fine wire mesh into 2 ml of isotonic saline. The cell suspension was centrifuged in the cold at 3000 rpm for 10 min; the supernatant was discarded and the sediment which contained approximately 0.05 ml of packed lymphoid cells was resuspended in 1 ml of isotonic saline. Lymphoid cells thus prepared were coupled to fraction II human γ-globulins in the following manner: 2 ml of human γ-globulin dissolved in isotonic saline (1 mg/ml) was added to 1 ml of lymphoid cell suspension, then after thorough mixing of these 2 components, 0.5 ml of BDB (1:15 dilution in phosphate buffer, pH 7.2) was introduced. The resultant mixture was maintained at room temperature for 10 min, after which it was centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the sediment was resuspended in 0.5 ml of isotonic saline. This procedure results in coupling of rat lymphoid cells to human γ-globulin. Nine adult Fischer rats were immunized. For immunization of animals, 0.5 ml of the suspension of coupled lymphoid cells was incorporated in 1 ml of Freund's adjuvant, and half this amount was injected intradermally, the rest i.m. Injections were repeated at 2-week intervals for a period of 3 months with a total of 6 injections. Nine adult Fischer rats were injected with autologous lymphoid cells combined with BDB and not coupled to human γ-globulin and served as the control series.

Since the object of the experiment was to study the amount of β-galactosidase in reticulum cells in spleen, thymus, mesenteric and axillary lymph nodes in the test and control animals, the spleen, thymus and mesenteric and axillary lymph nodes were removed and cut into blocks 2–3 mm thick. The blocks were placed on filter paper, inserted into glass tubes, immersed in a Dewar Flask containing acetone and dry ice and quickly frozen

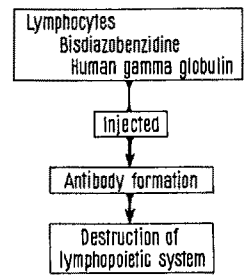

Fig. 1. The principle of immune destruction of the lymphopoietic system.

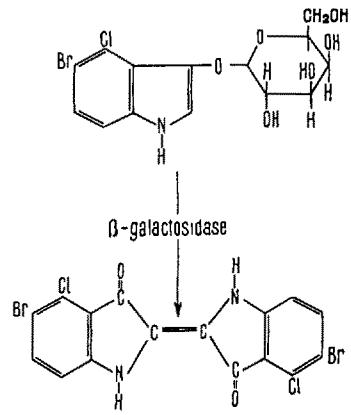

Fig. 2. Principle of the reaction for β-galactosidase.

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Galactosidase cells in lymphatic system

	Normal		Antigen (lymphocyte-protein complex used as antigen)
Spleen	(9) 14.6 ± 6.8 ± 2.3	$t = 7.259$ $p < 0.001$	(9) 109.7 ± 38.8 ± 12.9
Thymus	(9) 2.9 ± 3.3 ± 1.1	$t = 1.600$ $p < 0.20$	(9) 9.3 ± 11.5 ± 3.8
Mesenteric node	(9) 3.8 ± 2.3 ± 0.9	$t = 2.979$ $p < 0.02$	(9) 18.1 ± 14.2 ± 4.7
Axillary node	(9) 3.8 ± 2.6 ± 0.9	$t = 1.807$ $p < 0.10$	(9) 14.1 ± 12.6 ± 5.6

at − 70 °C. Frozen sections were cut at 6 μ in a Linderstrom-Lang Cryostat at − 20 °C and placed on a glass slide. The tissue frozen sections were fixed for 15 min in hydroxyadipaldehyde and placed in Coplin jars in an incubating mixture which contained: (1) 14.75 ml of 0.1 M pH 5.4, acetate buffer, (2) 0.25 ml NaCl having 0.015 M as the final concentration in 16 ml, (3) 1.0 ml substrate I (substrate I is 5-bromo-4-chloroindol-3-yl-β-D-galacytopyranoside) (4.1 mg/ml DMF) having 0.00063 M as the final concentration in 16 ml, and (4) 4.0 mg spermidine trihydrochloride being 0.001 M in 16 ml. Slides were incubated for $3^1/_2$ h at 27 °C, washed, dehydrated and coverslipped in permount. At this point all groups were statistically examined for galactosidase activity in the reticuloendothelial system using a microscopic grid of 490 constant squares employing FISCHER's Random Numbers. The values were statistically evaluated and all were significant.

The results of β-galactosidase activity in the reticulum cells of reticuloendothelial system are tabulated in the Table and in Figure 3. The only cells in the reticuloendothelial system showing the enzyme were the reticulum cells. The reticulum cells of the spleen show the highest β-galactosidase activity compared to the other reticuloendothelial organs. The histochemical enzyme reactions in lymph nodes was present in reticulum cells in the peripheral sinus and hilum. A small number of reticulum cells in the thymus gave a positive reaction. The activity of β-galactosidase is markedly increased when the lymphopoietic system is immunologically destroyed utilizing an autologous lymphocyte human globulin complex. The β-galactosidase activity in spleen was approximately 7 times greater than the activity in control animals. Furthermore, in addition, β-galactosidase activity was 3–4 times greater in thymus, mesenteric lymph nodes and axillary lymph nodes in the test animals over that of control animals. When the lymphopoietic system is immunologically destroyed, there may be an unmasking or proliferation of reticulum cells to regenerate this organ. These reticulum cells contain an abundant number of lysosomal enzymes such a β-galactosidase. Thus, the increase in lysosomal β-galactosidase is probably the result of increased number of reticulum cells. However, we do not feel that the increase in β-galactosidase resulted in similar manner as in our recent experiments where increase in β-galactosidase was achieved in the mammalian reticuloendothelial system utilizing an inducer substance[8,9].

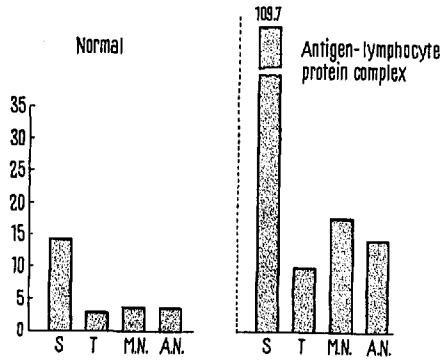

Fig. 3. Distribution of β-galactosidase cells in lymphatic system. S, spleen, T, thymus, M.N., mesenteric node, A.N., axillary node.

Zusammenfassung. Eine auffallende Zunahme von β-Galactosidase kommt im lymphoetischen Säuger-Gewebe im Zusammenhang mit der immunologischen Auflösung dieses Systems vor. Die hernach auftretende Proliferation retikulärer Zellen steht im Zusammenhang mit der Regeneration dieses Organs. Diese Zellen enthalten eine reichliche Anzahl lysosomatischer Enzyme (z.B. β-Galactosidase).

P. L. WOLF, J. P. HORWITZ, M. S. PAK, JANICE VAZQUEZ and ELISABETH VON DER MUEHLL

Detroit Institute of Cancer Research Division of the Michigan Cancer Foundation, Department of Pathology, Wayne State University, School of Medicine, and Detroit General Hospital, Detroit (Michigan 48207, USA)

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