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TECHNICAL MANUSCRIPT 504

COMPARATIVE STUDY FOLLOWING INJECTION OF AUTOLOGOUS LYMPHOCYTE BISDIAZOTIZED BENZIDINE FOREIGN PROTEIN

Alfred C. Standen
Bjarne Pearson

FEBRUARY 1969

DEPARTMENT OF THE ARMY
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Alfred C. Standen

Bjarne Pearson

Pathology Division
MEDICAL SCIENCES LABORATORIES

Project 1T061101A91A

February 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Autologous lymphocytes from mesenteric lymph nodes of adult New Zealand rabbits were coupled with 7S human gamma globulin and bisdiazotized benzidine (LGB) and injected into two groups of rabbits. Eight rabbits (group 1) were injected intravenously with 0.05 ml of LBG complex and sacrificed on the 10th day. Ten rabbits (group 2) received 0.05 ml LGB suspended in 1 ml incomplete Freund's adjuvant. One half of this suspension was injected intradermally, the remaining amount intramuscularly. This group was sacrificed 7 days following the injection. Group 3 consisted of 35 normal uninjected control animals. Areas of symmetrical lymphoid follicles of the spleen were compared in the two groups with the normal controls. The first group showed a significant difference in the size of splenic follicles in both the central and marginal zones as compared with the controls ($P = 0.001$). A significant difference was not observed in group 2. Differences between the two groups indicate that intravenous injection is preferred. Histochemical studies were made on all lymphoid tissues for β-galactosidase using indolyl substrate, and galactosidase-positive cells were counted. A significant increase occurred in the number of cells in group 1. This increase was marked in the thymus and popliteal lymph node and differs from previous results for specific β-galactosidase induction by isopropyl-β-D-thiogalactoside, as the latter effect subsided by the 7th day.

COMPARATIVE STUDY FOLLOWING INJECTION OF AUTOLOGOUS LYMPHOCYTE BISDIAZOTIZED BENZIDINE FOREIGN PROTEIN*

Early changes in the lymphatic system following injection of foreign protein have not been studied to any great extent, as most of the studies were done after the 7th day following multiple injections. These studies showed plasma cell proliferation. The paucity of studies during the early phase prompted us to investigate this problem. When 7S human gamma globulin or Cohn's fraction II were injected into New Zealand rabbits, certain changes were mainly apparent in the splenic follicles following human 7S gamma globulin injection.**

Symmetrical lymphatic follicles in the spleen were measured and their areas were calculated. In the control series these areas measured 0.15227± 0.012 mm^2 for the marginal zone and 0.08527±0.004 mm^2 for the central zone. Animals were sacrificed at 4, 24, 48, 72, and 96 hours and 7 days. The 7S human gamma globulin produced significant changes with decrease in the area of the marginal zone until 72 hours, followed by an increase through the 7th day. The central zone showed significant increases only at 72 and 96 hours. Both qualitative and quantitative cellular changes indicated involvement of polymorphonuclear cells at least during the early phases. Fully developed typical plasma cells were absent or very sparse. Cohn's fraction II showed no statistically significant changes either in areas of the marginal or central zones.

It seemed, therefore, that 7S gamma globulin could be used as a haptene if coupled to autologous lymphocytes with bisdiazotized benzidine. In this way early changes in the lymphatic system could be studied with the animal's own lymphocytes. Adult New Zealand rabbits were anesthetized with Fluothane,*** and laparotomies were performed. Portions of mesenteric lymph nodes were removed and divided into several pieces which were frozen for histochemical studies or preserved in alcoholic formalin. The remaining tissue was used for injection into each autologous donor. The fresh tissue was macerated through a fine wire mesh to separate lymphocytes from connective tissue. The lymphocytes were then suspended in 2 ml normal saline and centrifuged in the cold for 10 minutes. The supernatant was discarded, and the sediment, which contained about 0.05 ml of packed lymphatic cells, was resuspended in 1 ml normal saline.

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** Pearson, B.; Standen, A.C. 1966. Unpublished data.

*** Ayerst Laboratories, New York, New York.

To this suspension were added 2 ml of 7S human gamma globulin dissolved in normal saline (1 ml/ml). The solution was thoroughly mixed, and 0.5 ml of bisdiazotized benzidine (BDB) in a 1:15 dilution of phosphate buffer (0.2 M, pH 7.2) was introduced. The solution was maintained at room temperature for 10 minutes followed by centrifugation for 10 minutes in the cold. The supernatant was discarded, and the sediment was resuspended in normal saline.

The resulting complex (lymphocytes, 7S human gamma globulin, and bisdiazotized benzidine) (LGB), when injected into the donor rabbit, forms an autologous haptenized compound. The first group was injected intravenously with 0.05 ml of LGB. Group 2 received 0.05 ml of LGB suspended in 1 ml of incomplete Freund's adjuvant; one half of this suspension was injected intradermally, the other half intramuscularly.

The lymphatic system was examined histologically, and, in addition, quantitative measurements of the symmetrical splenic follicles were calculated for area including both the marginal and the central zones. Group 1 was sacrificed at 10 days and group 2 at 7 days following the initial injection of LGB.

β-Galactosidase activity was examined histochemically by the use of the indolyl substrate. A significant increase in the number of β-galactosidase-positive cells occurred in group 1 but not in group 2 when compared with the normal control animals. This increase was most marked in the thymus and popliteal lymph node and differs from previous results for specific β-galactosidase induction by isopropyl-β-D-thiogalactoside, which subsides by the 7th day.

Histological examination showed marked diminution of lymphocytes in the lymphatic system, which was particularly pronounced in group 1. Quantitative studies of the spleen of both groups are presented in Table 1. The marginal zone of group 1 shows a marked diminution in area, compared with the normal controls, with an area of 0.05048 mm^2 giving a P of 0.01 to 0.001. In group 2, however, the area measured 0.12130 mm^2 with a P of 0.5 to 0.3.

The central zone also showed a marked diminution in area, measuring 0.03760 mm^2, giving a P of <0.001 in group 1, whereas group 2 showed an area of 0.09250 with a P of 0.7 to 0.5. This is also shown in Table 1.

The results indicate that LGB complex, which contains autologous lymphocytes when injected into the donor host, may be capable of lymphocytic depletion. However, group 2 does not show significant changes. The differences between the two groups indicate that the intravenous injection is preferred. Absorption and possible resulting reactivity are definitely delayed in other routes of injection and when an adjuvant is incorporated in the injection LGB complex.

TABLE 1. COMPARISON OF SPLENIC FOLLICLE AREAS OF AUTOLOGOUS LYMPHOCYTE INJECTED RABBITS WITH NORMAL UNINJECTED ANIMALS

Group	Number	Marginal Zone		Central Zone	
		$\bar{x}$, mm^2	P	$\bar{x}$, mm^2	P
Control	35	0.15227	-	0.08527	-
1	8	0.05048	0.01 to 0.001	0.03760	<0.001
2	10	0.12130	0.5 to 0.3	0.09250	0.7 to 0.5

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13. ABSTRACT

Autologous lymphocytes from mesenteric lymph nodes of adult New Zealand rabbits were coupled with 7S human gamma globulin and bisdiazotized benzidine (LGB) and injected into two groups of rabbits. Eight rabbits (group 1) were injected intravenously with 0.05 ml of LGB complex and sacrificed on the 10th day. Ten rabbits (group 2) received 0.05 ml LGB suspended in 1 ml incomplete Freund's adjuvant. One half of this suspension was injected intradermally, the remaining amount intramuscularly. This group was sacrificed 7 days following the injection. Group 3 consisted of 35 normal uninjected control animals. Areas of symmetrical lymphoid follicles of the spleen were compared in the two groups with the normal controls. The first group showed a significant difference in the size of splenic follicles in both the central and marginal zones as compared with the controls ($P = 0.001$). A significant difference was not observed in group 2. Differences between the two groups indicate that intravenous injection is preferred. Histochemical studies were made on all lymphoid tissues for β-galactosidase using indolyl substrate, and galactosidase-positive cells were counted. A significant increased occurred in the number of cells in group 1. This increase was marked in the thymus and popliteal lymph node and differs from previous results for specific β-galactosidase induction by isopropyl-β-D-thiogalactoside, as the latter effect subsided by the 7th day. ( )

14. Key Words

*Lymphocytes
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