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The Immunohistological Changes Induced By Alloxan On The Pancreas Of Albino Rats

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ABSTRACT

The study of immnohistological changes in the pancreas induced by the administration of diabetogenic dose of alloxan was undertaken using male Albino (Wistar) rats. The rats were randomly separated into four groups A, B, C, and D of thirty rats in each group. The first group (A) was used as the primary control group, the second group (B), the third group (C) and the fourth group (D) were used as test groups. The test groups were administered with 200mg per kg body weight of alloxan, which is the diabetogenic dose. The second group (B) was used to study the direct effect of alloxan on the pancreas. The third group (C) was used to study the effect of diabetes mellitus on the pancreas while the fourth group (D) was used to study the possible effect of insulin therapy in the resuscitation of the pancreas. Examination of the pancreas revealed that they were affected in the test groups when compared to the control. The immunohistological features of the pancreatic islets were affected by alloxan administration, which showed selective toxicity for the islet beta cells as seen in the degeneration of these cells, while the other Islet cells were unaffected. This showed that the administration of diabetogenic dose of alloxan has a selective degenerative effect on the Beta cells of the islets of Langerhans, leading to immunohistological changes in the pancreas.

Key Words: Immunohistology, Alloxan, Islet cells, Pancreas, Albino rats.

Alloxan is a crystalline substance also called mesoxalyl urea, which is an oxidative product of uric acid (Sollmann, 1957; Bowman & Rand, 1985). Its parenteral injection into experimental animals causes degenerative lesions in various organs but with proper dosage, it produces prompt and highly selective coagulative necrosis of the beta cells of the Islets of Langerhans, resulting in a syndrome resembling clinical diabetes (Barnes & Eltherington, 1964; Bowman & Rand, 1985; Govindarajan, et al.; 2001) while injury to other tissues require larger doses. Following alloxan administration, the beta cells develop marked swelling then disappear resulting in diabetes while the alpha cells are not affected (Guyton & Hall, 1996; Samuelsson, et al., 2001). It has been shown that alloxan has proved to be the most convenient means of producing insulin deficiencies in laboratory animals by causing selective necrosis of the B-cells of the Islets of Langerhans even though the mechanism of action is not fully understood (Pasmore & Robson, 1970; Steiner & Freinkel, 1972; Bowman & Rand, 1985; Halfner, et al., 1996).

Antibodies to islet tissues have been demonstrated in newly diagnosed type 1 diabetes but these antibodies are not persistent and gradually disappear (Sameulsson, et al., 2001). These findings suggest that the antibodies are released temporarily as part of an antigen-antibody reaction associated with active destruction of the islet cells at the onset of diabetes (Bloom and Ireland, 1980; Bowman & Rand, 1985; Alberti & Krall, 1991; Sell, 1978). The presence of permanent antibodies to islet cells, confirms that in these cases, diabetes is merely one aspect of a primary immune disorder in which the islet cells are destroyed by unprovoked antibody reaction (Alberti & Krall, 1991; Kumar & Clark, 1991; Edwards, et al., 1995; Weetman, 1991; Stites, et al., 1994; Govindarajan, et al., 2001).

It has been shown that the beta cells of the Islets of Langerhans of pancreas, develop marked swelling before final disappearance while the detailed

immunohistological sequence of events leading to the disappearance of these islets of pancreas during the induction of diabetes mellitus has not been fully elucidated. The present study is aimed at investigating the immunohistological changes leading to the disappearance of the islet beta cells following induction of diabetes mellitus in rats.

MATERIALS AND METHODS

Alloxan crystals contained in a 250gm bottle was used for the work. The alloxan crystals weighing 1.2gm were dissolved in 10ml of injection water, amounting to 1.2gm/10ml. Then, 0.3ml of the 1.2gm/10ml solution of alloxan, which is equivalent to 40mg of alloxan was administered as the diabetogenic dose according to Barnes & Eltherington, (1964); Barbato & Landau, (1977). Bowman & Rand, (1985); Greenspan & Baxter, (1994).

The test animals were 120 male Albino (Wistar) rats, which were randomly separated into four groups, A, B, C and D of thirty rats in each group. The first group (A) was used as the control group. The second group (B), the third group (C) and the fourth group (D) were used as the test groups and as such were administered with 0.3ml of 1.2gm/10ml solution of alloxan, equivalent to 200mg/kg body weight which contained 40mg of alloxan. This single diabetogenic dose of alloxan was administered to the test animals through the intraperitoneal route (IR) while the control group received normal saline through the same route.

The group B animals were the first test groups and were used to study the direct effect of alloxan on the pancreas. The second test group (C) are animals that have been induced with diabetes mellitus and were used to study the effect of diabetes mellitus on the pancreas. The third test group (D) were diabetic animals undergoing insulin therapy and were used to study the possible effect of the therapeutic agent on the resuscitation of the pantreatic Islets of Langerhans.

Tissue Preparation For Microscopy

Animals from each of the groups were anaesthetized and their abdomen opened by midline incision and the pancreas from each of the groups were excised. The tissue were fixed in 10% buffered formalin and Bouins fluid. The tissues were processed using automatic tissue processing machine called the Histokinette bench model tissue processor obtained from the Dept of Human Anatomy, ABU Zaria.

The tissues were embedded in paraffin wax and sections of about. 8 microns were made using Rotary microtome. The paraffin-embedded tissue sections were deparaffinized in two changes of xylene for 5 minutes each. The tissues were rehydrated using absolute ethyl alcohol, 90% and 80% ethyl alcohol. They were rinsed gently in running tap water for 30 seconds and treated with 3% hydrogen peroxide solution. The tissues were incubated at room temperature for 5 minutes, rinsed with phosphate buffered saline (PBS) and allowed in PBS for 2 minutes.

Primary antibody was incubated with the tissue for 60 minutes at 370C and rinsed with PBS. The tissues were placed in PBS for 5 minutes and peroxidase conjugated secondary antibody were diluted and applied to the tissues. The tissues were incubated for 30 minutes at room temperature, rinsed in PBS and placed in PBS for 5 minutes. The tissues were counterstained using Mayers hematoxylin according to the methods of Leduc and Avrameas, (1970); Culling (1993); Ikuno, et al., (1995) Sigma, 1998 and Samuelsson, et al.; (2001) called the immunoperoxidase staining method, which is used to demonstrate immune complex reactions within tissues.

RESULTS

The results of the microscopic examination of the immunoperoxidase staining of the pancreas, show normal isolated pancreatic Islets of Langerhans with pancreatic Islet cell antibodies in all the Islet cell types as shown in Fig.1. The results, also show the presence of antibodies to alpha cells (A), delta cells (D) and pancreatic polypeptide cells (PP) which were used to demonstrate the presence and viability of these cells and their ability to produce their hormones for secretion. This leaves the Beta cells negative to these antibodies after alloxan administration during the induction of diabetes mellitus, during diabetes mellitus stage and during insulin therapy as shown in Figs. 2, 3 and 4 respectively. These changes in the immunohistology of the pancreas were collaborated with the changes in the blood

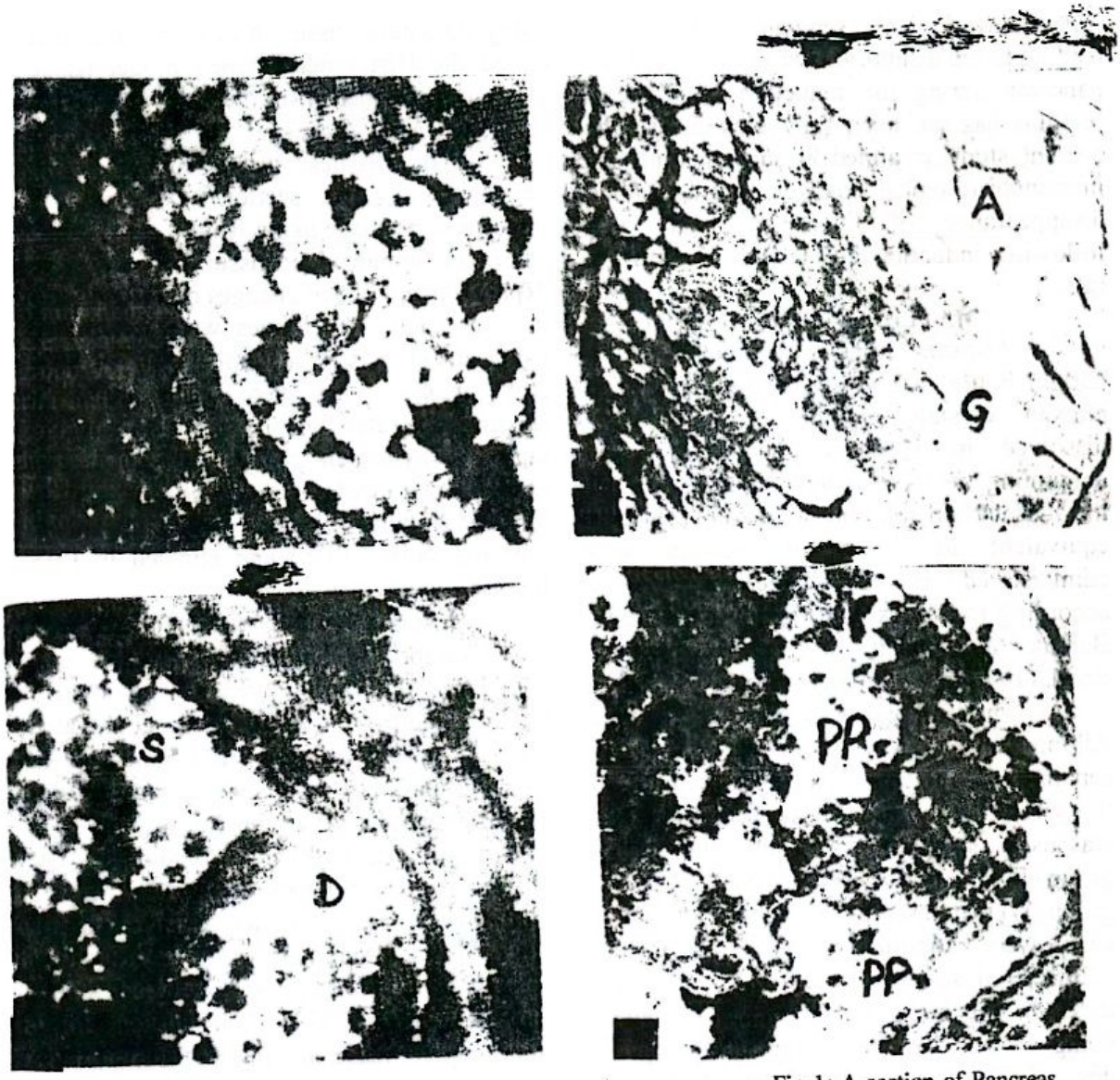

Fig 1: A section of Pancreas from the control (A) group, showing the presence of Pancreatic Islet cell antibodies in all Islet (I) Cell types. Immunoperoxidase x 400.

Fig 2: A section of Pancreas from group B, showing the Islet and antibodies to glucagon (G). This demonstrates the presence of antibodies to alpha (A) cells after alloxan administration. Immunoperoxidase x 400.

Fig 3: A section of Pancreas from Group C, showing Islet with antibodies to Somatostatin (S). This demonstrates the presence of antibodies to delta (D) cells after alloxan administration. Immunoperoxidase x 400.

Fig 4: A section of Pancreas from group D, showing Islet with antibodies to pancreatic polypeptide (PP) to demonstrate the presence of antibodies to PP cells after alloxan and insulin administration. Immunoperoxidase x 400.

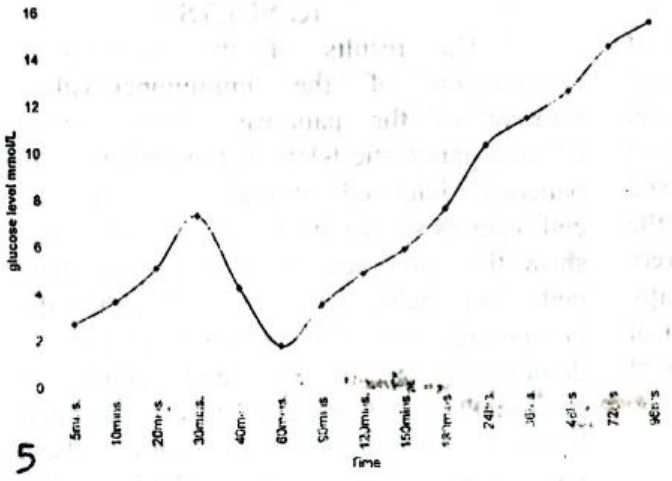

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Fig. 5 Effect of alloxan on the blood glucose level in rats

glucose level of the rats, which indicate hyperglycaemia as shown in Fig 5.

DISCUSSION

The result of alloxan administration in rats show changes in the immunohistological features of the pancreas. It has been shown that the first reaction to occur after alloxan administration is inflammation of which are infiltration of cells into the pancreatic tissues. The first cells to infiltrate the islets are macrophages followed predominantly by T-cells (Dean, et al., 1985; Walker, et al., 1988; Weetman, 1991; Samuelsson, et al., 2001). T-cell lines specific for islet cells antigen have been isolated from the pancreas and spleen of diabetic rats. (Weetman, 1991; Prud' homme, et al., 1984; Kolb, 1983).

T-cells have been shown to be important in the development of diseases (Plamondon, et al. ,1990). The potential role of Islet antibody (Ia) expression by pancreatic beta cells in stimulating T-cells was revealed by Ia positive cells stained immunohistochemically for insulin which showed to be phagocytic leucocytes with intracellular insulin from phagocytized and destroyed beta cells (Int'Veld and Pipeleers, 1988; Weetman, 1991; Govindarajan, et al., 2001).

Antibodies recognising islet cells surface molecules called the islet cell antigen and insulin are found in prediabetic phase and in majority of diabetic rats (Dyrberg, et al., 1984; Baekkeskov, et al., 1984; Laborie, et al., 1985; Dean, et al., 1987). It has been shown that there is little correlation between the antibodies, even though the Islet cells surface antibodies are strongly associated with the development of diabetes (Weetman, 1991). But it is also clear that diabetes may develop in some animals in the absence of any detectable antibodies (Dean, et al., 1987). This, as Weetman (1991) Baekkeskov, et al., (1984) asserted, could be due to assay insensitivity or presence of other pathogenic autoantibodies which may occur secondary to Islet cell damage. This is true because, Elder, et al., (1982) has demonstrated the presence of antibodies against thyroglobulin and gastric parietal cells in some diabetic rats. These diabetic animals also have lymphocyte antibodies of unknown significance but compatible with polyclonal activation (Dyrberg, et al., 1984). Martin & Logothetopoulos, (1984); Laborie, et al., (1985); Govindavajan, et al., (2001) have noted that islet cell surface antibodies fix complements and may be cytotoxic to beta cells but not alpha cells in vitro. It has been shown by more recent analysis relating such cytotoxicity to beta cell volume determined by biosy, that the appearance of these antibodies is a secondary reaction to on-going loss of beta cells (Hehmke, et al, 1990; Samuelsson, et al., 2001). The authors reasoned that humoral cytotoxicity is involved in the rapid destruction of the remaining beta cells after the onset of hyperglycaemia.

Sufficient dose ot stroptozotocin, an antineoplastic antibiotics, generally cause acute degeneration of beta cells whereas repeated smaller doses in certain strains of mice produce delayed but progressive increase in blood glucose, associated with insulitis and beta cell destruction (Appeal, et al, 1978; Kantwerk, et al., 1987). There is evidence to show that autoimmune responses against beta cells can be induced by a variety of viruses. Chemical toxins like streptozotocin or alloxan, produce similar forms of diabetes even though the histology in some cases is unlike that seen in a typical disease in man (Samuelsson, et al., 2001).

REFERENCES

Alberti KGM and Krall LP (eds.)(1991). The Diabetes Annual, 6. Elsevier Science Publishers, Amsterdam. PP. 2 - 6.

Appeal MC, Rossini AA, William RM and Like AA (1978). Viral Studies in Streptozotocin induced Pancreatic Insulitis. Diabetologia. **15**: 327:336.

Baekkeskov S, Dyrberg T and Lernmark A (1984). Autoantibodies to a 64 kilodalton Islet Cell Protein Precede the Onset of Spontaneous diabetes in the BB rat. Science. **224**: 1348 - 1350.

Barbato C and Landau M (1977). Drug induced endocrine disorder. Churchill Livingstone, London. PP. 420.

Barnes C D and Eltherington, L G (1964). Drug dosage in laboratory animals: A handbook. University of California Press. California. PP. 26.

Bloom A and Ireland J (1980). A colour Atlas of Diabetes. Wolfe Medical Publication, London. Pp. 4 - 20.

Bowman WC and Rand MJ (1985). Textbook of pharmacology. 3rd ed. Blackwell Scientific Publi. London, Pp. 1943 - 1964.

Culling CFA (1993). Handbook of Histological Techniques. 6th ed. Butterworths, London, PP. 120 - 130.

Dean BM, Walker R, Bone AJ, Baird JD and Cooke A (1985). Pre-diabetes in the Spontaneously diabetic BB/E rat: Lymphocyte subpopulations in the pancreatic infiltrate and expression of rat MHC Class II molecules in endocrine cells. Diabetologia. **28**: 464 - 466.

Dean BM, Bone AJ, Varey AM, Walker R, Baird JD and Cook A (1987). Insulin auto antibodies, Islet Cell surface antibodies and the development of spontaneous diabetes in BB Edinburgh rat. Clinical and Experimental Immunology. **69**:308 - 313.

Dyrberg T, Poussier P, Nakhoodsa F, Marliss EB and Lernmark A (1984). Islet Cell surface and lymphocyte antibodies often precede the spontaneous diabetes in BB rat. Diabetologia. **28**: 464 - 466.

Edwards CRW, Bouchier IAR, Haslett C and Chilvers ER (eds.) (1995). Davidson's Principles and Practice of Medicine. 17th ed. Churchill-Livingstone, NewYork. PP. 724 - 768.

Elder M, Maclaren N, Riley W and Mc Connell T (1982). Gastric parietal cell and other autoantibodies in the BB rats. Diabetes. **31**:313 - 318.

Govindarajan M, Mohan V, Deepa R., Ashok S and Pitchumoni CS (2001). Histopathology and Immnohistochemistry of Pancreatic Islets in fibrocalculous pancreatic diabetes. Diabetes Res-Clin-Pract. **51** (1): 29 - 38.

Greenspan A and Baxter O (1994). Basic and Clinical endocrinology. Appleton and Lange Cop. Standford. PP. 150 - 185.

Guyton AC and Hall EJ (1996). Textbook of Medical Physiology. 9th ed. W. B. Sauders company, London. Pp. 926 - 972.

Haffner SM, Miettinen H, Stern MP (1996): Insulin Secretion and resistance in non-diabetic Mexican Americans and non-Hispanic whites with a parental history of diabetes. Journal of Clinical Endocrinology and Metaboli: **81** (5): 1846 - 1851.

Hehmke B, Lucke S, Schroder D, Kloting I and Kohnert KD (1990). Complement dependent antibody - mediated cytotoxicity in the spontaneous diabetic BB/OK rat: association with B-cell volume density. European Journal of Immunology. **20**:1091 - 1096.

Ikuno BC, Miodovni K M, Rosenn BM Khour JC, Grigsby JL (1995). Pregnancy outcome in the diabetes control and complications trials. American Journal of Obstet and Gynecol. **174** (4): 1343 - 1353.

In't Veld PA. and Pipeleers DG (1988). In situ analysis of pancreatic islets in rats developing diabetes: Appearance of nonendocrine cells with surface MHC Class II antigens and cytoplasmic insulin immunoreactivity. Journal of Clinical Investigation. **82**: 1123 - 1128.

Kantwerk G, Cobbold S, Waldmann H and Dose H (1987). L3T4 and Lyt-2T-cells are both involved in the generation of low-dose streptozotocin -induced diabetes in mice. Clinical and Experimental Immunology. **70**:585 - 592.

Kolb H (ed) (1983). Diabetes and Immunotherapy. J. K. Burgess, Minneapolis. PP 1 - 108.

Kumar PJ and Clerk D (1991). Clinical Medicine: A Textbook for medical students and doctors. ELBS, London. PP. 832 - 858.

Laborie C, Sai P, Feutren G, Debray-Sachs, M, Quiniou-Debire MC, Poussier P, Marliss EB and Assan R (1985). Time course of Islet cell antibodies in diabetic and non-diabetic BB rats. Diabetes. **34**: 904 - 910.

Leduc EH and Avrameas J (1970). Cellular origins of Humoral antibodies. Triangle: The Sandoz Journal of Medical Science **9**(6):220 - 228.

Martins DR and Logothetopoulos, J (1984). Complement fixing Islet Cell antibodies in the Spontaneously diabetic BB rat. Diabetes **38**:1329 - 1331.

Passmore R and Robson JS (eds) (1970). A companion to medical studies. Vol. 2: Pharmacology, Microbiology, General Pathology and related subjects. Blackwell Scientific Publication, Oxford. Pp. 2513 - 2520.

Plamondon C, Kottis V, Brideau C, Metroz-Dayer MD and Poussier P (1990). Abnormal thymocyte maturation in spontaneously diabetic BB rats, involves the deletion of CD 4-8+ cells. journal of Immunology. **144**:923 - 923.

Prud'homme GJ, Fuks A, Colle E and Guttmann RD (1984). Isolation of T-lymphocytes lines with specificity for Islet cell antigens from spontaneously diabetic (Insulin-dependent) rats. Diabetes **33**: 801 - 803.

Samuelsson U, Sundkvist G, Borg H, Fernlund P, Ludvigsson J (2001). Islet autoantibodies in the prediction of diabetes in school children. Diabetes - Res - Clin - Pract. **51**(1): 51 - 57.

Sell S (1978). Immunopathology. American Journal of Pathology. **90**:211.

Sigma (1998). Immunochemicals. Catalogue of Sigma - Aldrich Comp. Ltd. England. PP. 373 - 431.

Sollmann T (1957). A manual of Pharmacology and its application to Therapeutics and Toxicology. 8th ed. W. B. Saunders Publishers, London. PP. 75 - 77.

Steiner DF and Freinkel N (eds) (1972). Handbook of Physiology, Section 7: Endocrinology Vol.1: Endocrine Pancreas. American Physiological Society, Washington D. C. PP. 1520 - 1580.

Stites DP, Terr J and Parslow MN (1994). Basic and Clinical Immunology. Lange Medical Publication, California. PP. 52 - 89.

Walker R, Bone AJ, Cooke A and Baird J (1988). Distinct macrophage subpopulations in the pancreas of prediabetic BB/E rats. Possible role for macrophages in pathogenesis of IDDM. Diabetes. **37**:1301 -1 - 1304.

Weetman AP (1991). Autoimmune endocrine disease - Cambridge Uni. Press, Cambridge. Pp. 163 - 210.