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THE EFFECTS OF CYCLOPHOSPHAMIDE AND THYMOPOIETIN  
ON THE CELL-MEDIATED IMMUNE RESPONSE  
IN THE GUINEA PIG

by

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Submitted to the Faculty of the Graduate School  
in partial fulfillment of the requirements  
for the degree Doctor of Philosophy  
in the Department of Anatomy  
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September, 1977

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## V. SUMMARY

Functional and histological evaluations of various tissues in the guinea pig were made to determine the effects of DNCB, cyclophosphamide, and thymopoietin on the cellular immune response. Quantitative assays included the skin reactivity at the site of challenge and the percentage of rosette forming T lymphocytes in the peripheral blood. Qualitative studies included the histological appearance of cell populations in the spleen, lymph nodes, and skin, by both light and fluorescence microscopy.

Forty-five experimental guinea pigs were sensitized and challenged with DNCB. The experimental groups consisted of normal, single dose cyclophosphamide, double dose cyclophosphamide, thymopoietin, and combined thymopoietin-cyclophosphamide. Experimental guinea pigs were evaluated for the mean area of the lesion and the amount of erythema and induration ten days after the challenge was administered. Five guinea pigs were used for controls. All animals were bled by cardiac puncture and were dissected for peripheral lymphatic tissue and skin ten days post challenge. Lymphocytes were separated from the peripheral blood and incubated with rabbit erythrocytes to form rosettes. Tissue prepared for light microscopy was fixed in buffered formalin and stained with hematoxylin-eosin. Frozen sections of spleen and lymph node were stained with FITC conjugated to rabbit anti-guinea pig IgG and examined by fluorescence microscopy.

Skin lesions of groups treated with a single dose of cyclophosphamide and a double dose of cyclophosphamide demonstrated an insignificant increase in the mean area of the lesion compared with the group receiving only DNCB. This slight enhancement of the contact dermatitis reaction was presumed to reflect the loss of inhibitory effects of B lymphocytes and their products. The mean area of the skin lesion in groups treated with thymopoietin and the combined therapy of thymopoietin-cyclophosphamide demonstrated significant increases compared with groups receiving only DNCB, a single dose of cyclophosphamide and a double dose of cyclophosphamide. This indicated the tremendous potential of thymopoietin to induce competence in populations of T lymphocytes which were then capable of responding to the immunogen.

Rosette forming cells were found to decrease in groups treated with only DNCB, a single dose of cyclophosphamide, and a double dose of cyclophosphamide, compared with the unsensitized and untreated control group. This might be explained by the probable mobilization of T lymphocytes originally located in the peripheral blood into tissues at the site of challenge, such as the skin and peripheral lymphatic tissue. The thymopoietin treated group showed a significant increase in the percentage of rosette forming cells compared with control and normal DNCB groups. This increase in the number of peripheral blood T lymphocytes

capable of interacting with rabbit erythrocytes indicated that thymopoietin was responsible for inducing competence in immature T lymphocytes. The group receiving a combined therapy of thymopoietin-cyclophosphamide demonstrated a reduction compared with the group treated with only thymopoietin. This was presumed to reflect the negative influence of cyclophosphamide on T lymphocytes in the peripheral blood that had been stimulated by thymopoietin.

Spleens of control guinea pigs demonstrated an abundance of white pulp with distinct periarterial lymphoid sheaths and marginal zones. When animals were treated with a single or double dose of cyclophosphamide, a loss of cells was observed in the marginal zone and the red pulp. These were confirmed to be B lymphocytes in tissues treated with FITC anti-guinea pig IgG which demonstrated a reduction in the numbers of fluorescing cells, indicating the negative effect of cyclophosphamide on mitostatic activity. Guinea pigs treated with thymopoietin showed an increase in the size of the periarterial lymphoid sheaths with normal appearing marginal zones and red pulp. This indicated that thymopoietin was also responsible for increasing the total number of cells available in peripheral lymphatic tissue. A combined therapy of thymopoietin-cyclophosphamide demonstrated a reduction in the lymphocytes of the marginal zone and the red pulp with an increase in lymphocytes of the periarterial lymphoid sheath. A decrease from the

normal population of immunoglobulin G-producing B lymphocytes in the marginal zone and the red pulp was confirmed by examination of tissues labeled with FITC anti-guinea pig IgG.

Lymph nodes demonstrated the same phenomenon as spleens. Control animals revealed a discrete cortex with numerous lymph follicles. When animals were treated with a single or double dose of cyclophosphamide, a partial to a complete loss of lymph follicles and B lymphocytes were observed by light and fluorescence microscopy respectively. Thymopoietin treated guinea pigs showed a normal appearing cortex with an increase in the density of small lymphocytes in the paracortex. Guinea pigs that received a combined treatment of thymopoietin-cyclophosphamide demonstrated a reduction of lymph follicles and fluorescing B lymphocytes found by light and fluorescence microscopy, with an increase in the density of small lymphocytes in the paracortex observed by light microscopy.

Skin of control animals revealed a thin epidermis and a dermis that contained fibrocytes and small lymphocytes. All experimental groups demonstrated a thickened epidermis and an infiltration of small lymphocytes into the dermis. This was an expected finding of the contact dermatitis reaction.

This experiment evaluated the effects of a known B lymphocyte suppressing agent, cyclophosphamide, and a

proposed T lymphocyte enhancing agent, thymopoietin, on the cellular immune response. It was found that cyclophosphamide had a slight enhancing effect on cellular immunity by depleting B lymphocyte populations. More noteworthy was the exaggerated influence of thymopoietin on the induction of competence in immature lymphocytes which were then capable of responding to the immunogen.

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