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Studies on the Secretory Immunological System of Fowl

II. IMMUNOGLOBULIN-PRODUCING CELLS ASSOCIATED WITH MUCOUS MEMBRANES

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Summary. Tissue sections from various areas of the digestive and respiratory tracts and spleens of chickens were examined by the fluorescent antibody technique for the presence of immunoglobulin-containing cells. All tissues examined contained lymphoid cells staining for immunoglobulin with the duodenum and caecal tonsils containing the highest concentrations of such cells. Spleens had approximately equal numbers of IgM- and IgY-containing cells; other tissues showed a predominance of IgY-containing cells.

The distribution of IgY-containing cells in the chicken gastrointestinal tract was similar to that generally associated with the secretory immunologic system of mammals which shows a preponderance of IgA-containing cells. These results in conjunction with other available data suggest that the chicken has evolved a secretory immunological system wherein the predominant serum immunoglobulin and the secretory immunoglobulin belong to the same class.

INTRODUCTION

Chickens can develop a high level of resistance to Newcastle disease virus following the intranasal inoculation of vaccines (Lancaster, 1964). Similar observations have been made following aerosol vaccination (Lancaster, 1964; Beard and Easterday, 1967). These observations in conjunction with the histological data available on the distribution of lymphoid tissues in chickens (Thorbecke *et al.*, 1957; Bang and Bang, 1968; Janković and Mitrović, 1967) suggest that chickens may possess a secretory immunological system similar to that of man (Tomasi and Bienenstock, 1968).

It is now well established that chicken serum contains 19S and 7S immunoglobulins. The former immunoglobulin closely resembles mammalian IgM whereas it has been suggested that the chicken serum 7S immunoglobulin more closely resembles human IgA than IgG (Tenenhouse and Deutsch, 1966). Recent studies (Leslie and Clem, 1969, 1970; Mehta and Tomasi, personal communication) have indicated differences between normal human IgG and normal chicken 7S immunoglobulin. On the basis of these data and the unusual reduction characteristics of several gallinaceous bird immunoglobulins (Dreesman and Benedict, 1965; Leslie and Benedict, 1969), we proposed the interim designation of IgY for the predominant 7S serum immunoglobulin (Leslie and Clem, 1969; Clem and Leslie, 1969).

Most immunoglobulin in chicken secretions is IgY, with trace amounts of IgM (Leslie, Wilson and Clem, 1971). The following immunofluorescent and histological investigations were made with tissues from chickens in order to study the distribution of immunoglobulin-producing cells, and ascertain the class of immunoglobulins contained in lymphoid cells in areas associated with a secretory immunological function.

MATERIALS AND METHODS

Animals

Mature white Leghorn birds were used.

Preparation of immunoglobulins and antisera

The preparation of chicken IgM and IgY, their component polypeptide chains, and antisera to heavy (H) chains, light (L) chains and whole chicken serum has been described (Leslie and Clem, 1969).

Immunofluorescent and histological procedures

The technique for preparation of the immunofluorescent reagents from specific antisera was that of Wood, Thompson and Goldstein, 1965. Tissue preparation and staining procedures have been described previously (Crandall, Cebra and Crandall, 1967). Tissue sections were stained directly for immunoglobulin-containing cells with fluorescein labelled rabbit anti-chicken L chain, anti Y chain or anti μ chain. In certain cases, the indirect fluorescent antibody technique was used for IgY localization employing rabbit antisera specific for chicken IgY and fluorescein labelled goat anti-rabbit IgG. Specificity of the immunofluorescent reagents was tested by blocking tests with the appropriate isolated immunoglobulin. Only the IgM reagent required repeated absorption to achieve specificity.

Frozen sections of the same areas examined for immunofluorescence were stained with haematoxylin and eosin and examined by light microscopy. The lymphoid areas of the tissues studied have been described (Thorbecke *et al.*, 1957; Heuschele and Easterday, 1970b; Janković, 1968) and our histological observations essentially agreed with these published descriptions.

RESULTS

Our initial objective was to confirm the distribution of immunoglobulin-containing cells in the chicken. For this we used fluorescein-labelled anti-chicken L chain reagents. Fluorescent cells were present among the lymphoid cells of the duodenum, ileum, colon, caecal tonsils, bronchus, trachea and in the spleen. Typical examples of immunoglobulin-containing cells in some of these tissues are shown in Fig. 1. There was marked variation in the number of stained cells found in sections of similar tissues from the four different animals examined. However, the gastrointestinal tract always contained large numbers of immunoglobulin-containing cells and the greatest number of these cells were located in the lamina propria of the duodenal mucosa and in the caecal tonsils. Only a few immunoglobulin-containing cells were observed in the colon.

An evaluation of the number of fluorescent cells along the respiratory tract presented some technical problems due to the mucous present. Definite staining of cells with plasma

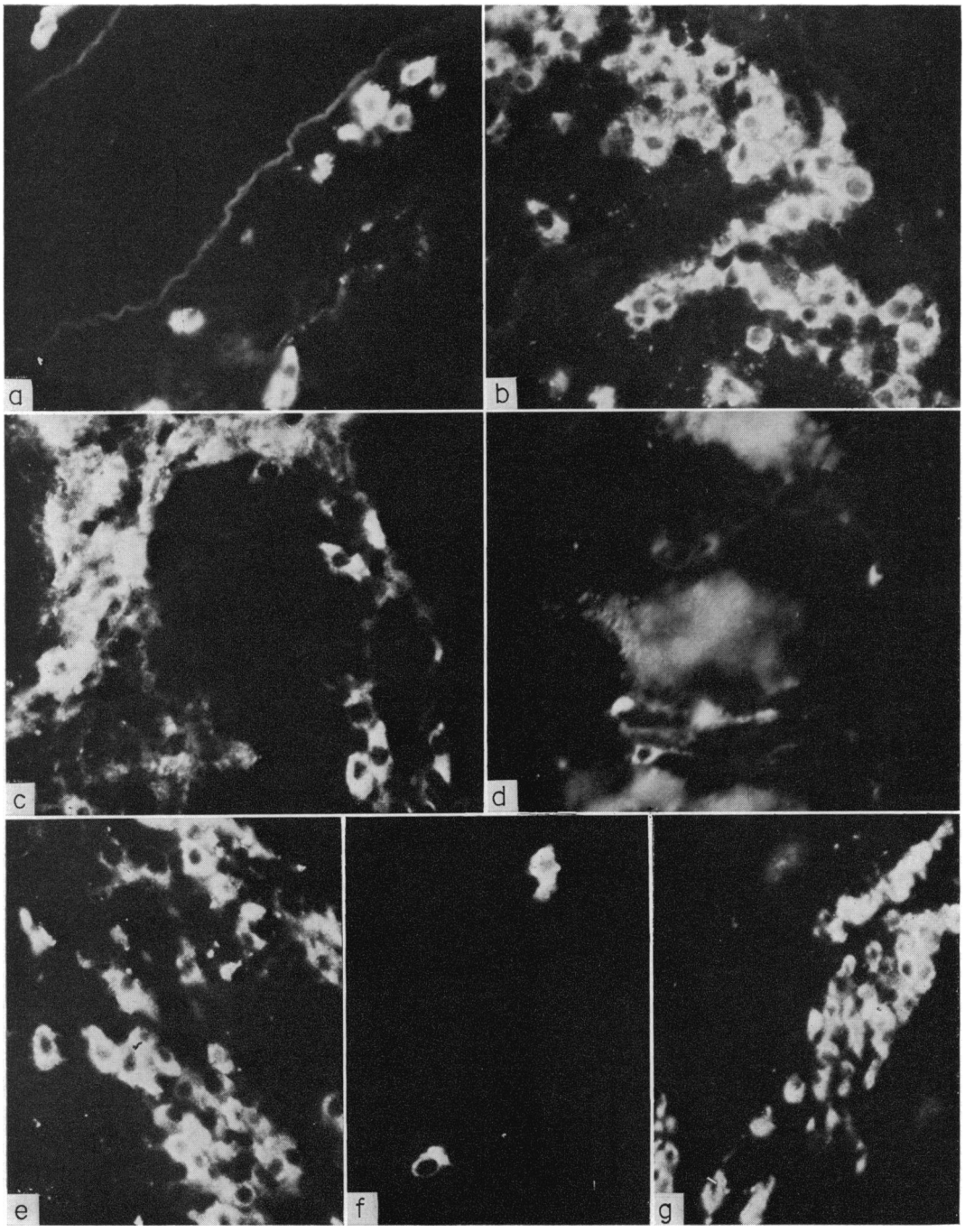

FIG. 1. Photomicrographs of immunoglobulin-containing cells in chicken tissues. a–d stained with chicken anti-light chain. (a) Ileum; (b) Caecal tonsil; (c) Bronchus; (d) Trachea; (e) Duodenum stained with anti-light chain; (f) Duodenum stained with anti-μ chain; (g) Duodenum stained with anti-y chain.

cell morphology was seen in sections of the bronchus and trachea (Fig. 1c, d). In addition, many areas along the respiratory tract showed a diffuse homogeneous or sometimes granular staining pattern, which we attribute to the presence of immunoglobulin in mucous glands and mucous present on the surface of the epithelium.

Having established that immunoglobulin-containing cells were present in areas associated with a secretory immunological function we focused our attention on the classes of immunoglobulins present in these cells. In three out of four birds there was no doubt that the IgY-containing cells greatly outnumbered the IgM-containing cells in the tissues examined with the exception of the spleen where they were about equal. An example of the frequency of the cells containing the different classes of immunoglobulins in the duodenal mucosa is shown in Fig. 1e, f, g. It must be stressed that this Figure does not imply that IgM-containing cells are rare along the mucous membranes. The fourth bird that we examined was very unusual in that only a few cells could be detected in the spleen with anti-L chain reagent and the majority of the gastro-intestinal plasma cells were of the IgM type. Unfortunately other tissues from this animal were not examined. Semi-quantitative estimates of the various types of plasma cells along the digestive tract showed that there was at least three times as many IgY-containing cells as IgM-containing cells.

Since our data are only semi-quantitative it does not rule out the existance of immunoglobulin classes other than IgY and IgM. However, based upon counts of twenty microscopic fields each for anti-L, anti-Y and anti-μ in the duodenal mucosa, it is clear that the sum of the IgY and IgM-staining cells account for at least the majority of the immunoglobulin-containing cells stained with anti-L chain reagent. The existence of a minor immunoglobulin class could not be ruled out.

DISCUSSION

Our previous studies have demonstrated that IgY is the predominant immunoglobulin in the secretions of chickens (Leslie *et al.*, 1971). Heuschele and Easterday (1970a and b) have also reported the presence of globulins in surface mucus of the chicken trachea and the presence of globulin-containing lymphoid cells in the tracheal mucosa; they also demonstrated the synthesis and secretion of specific antibody by the trachea. Neither of these latter studies characterized the class of chicken immunoglobulins in the secretions and the cells of the tracheal mucosa.

Our immunofluorescent studies clearly demonstrate that in the chicken large numbers of immunoglobulin-containing cells populate the lamina propria of the gut wall, especially in the duodenum and caecal tonsil. Likewise, the trachea and bronchi contain immunoglobulin-containing cells. Although considerable variation was seen, the predominant immunoglobulin in the cells of these areas was IgY. The results are similar to those of Kincade and Cooper (1970). In one animal virtually all of the cells contained IgM. Unfortunately no information was available on the serum proteins of this animal and it may in fact have represented a myeloma-like condition.

Does the chicken possess a secretory immunological system? On the basis of the presence of one predominant immunoglobulin in the respiratory, gastrointestinal and genital secretions, the distribution of immunoglobulin-containing cells, and the local production and secretion of specific antibody by the trachea (Heuschele and Easterday, 1970a, b; Leslie *et al.*, 1971), we feel that the evidence is strongly in favour of such a system. The problem then becomes one of trying to relate the structure of an established secretory IgA

molecule, such as that reported for man, rabbit and dog, to that of the IgY found in chicken secretions. We have been unable to demonstrate an additional secretory component on secreted IgY. On occasion very small amounts of 'heavy' IgY (eluted in first fractions from Sephadex G-200) have been seen in secretions but the ease with which serum IgY aggregates makes premature any statement relative to the significance of this 'heavy' IgY in secretions. The distribution of IgY-containing cells is similar to that of mammalian IgA-containing cells (Tomasi and Bienenstock, 1968).

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