New Immunoglobulin-like Molecules in the Serum of Bursectomized–Irradiated Chickens

(heavy(μ)chain/immunoelectrophoresis/agammaglobulinemia)

YONG SUNG CHOI AND ROBERT A. GOOD

The Pathology Research Laboratories, Variety Club Heart Hospital and Department of Pediatrics, University of Minnesota, Minneapolis, Minn. 55455

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ABSTRACT A new immunoglobulin-like protein that was found in the sera of bursectomized and irradiated chickens is reported. Unlike IgM and IgG, this protein is apparently made only of heavy chains of IgM and contains no light chains.

When newly hatched chickens are bursectomized and irradiated, they fail to synthesize circulating immunoglobulins, even though the expression of cellular immunity apparently remains normal (1, 2). In the chicken, the bursa of Fabricious is essential for the development of lymphoid cells that will synthesize and secrete both IgM and IgG immunoglobulins.

The present report describes a new immunoglobulin-like protein present in the sera of such agammaglobulinemic chickens. This protein, which is found in the absence of IgM and IgG, apparently has a structure different from any reported immunoglobulin. It is made only of heavy chains of the μ-type and contains no light chains.

MATERIALS AND METHODS

Bursectomy and irradiation

The inbred strain of white Leghorn chickens, line 91, was obtained from Hyline Poultry, Inc.

Chicken embryos were bursectomized in ovo at 18 days of embryonation and irradiated on the day of hatching, as described by Cooper et al. (3) Newly hatched chickens were exposed to sublethal doses of total body x-irradiation at a dose rate of 48.2 R/min in air, to a total dose of 650 R. For the most effective irradiation, the chickens were packed in a specially designed lucite cage that was divided into two concentric rings, which were each subdivided into 20 wedge-shaped compartments. Each compartment holds one newly hatched chicken.

Antisera

Rabbit antisera against chicken immunoglobulins were prepared by injection of purified chicken IgM and IgG; For immunization, chicken IgM and IgG were prepared as follows: chickens were stimulated by injections of Dnp-Brucella, about 10¹⁰ organisms/chicken, in complete Freund adjuvant. Immune chicken serum was adsorbed by Dnp-Sephoreso and eluted by 0.2 M glycine-HCl buffer (pH 2.8), as described by Cuatrecases et al. (4). IgM was separated from IgG by gel filtration through a Sephadex G-200 column (2.5 × 100 cm) in 0.015 M Tris·HCl buffer (pH 7.4) with 0.14 M NaCl. Chicken immunoglobulin thus purified exhibited a single band in immunoelectrophoresis against a rabbit antiserum prepared against whole chicken serum. The purity of IgM and IgG was further verified by demonstrating a single band in rabbit antiserum prepared against these purified antigens.

Anti-μ antiserum was prepared from anti-IgM by a solid immune adsorption technique with polymerized chicken IgG by the method of Avrameas and Ternynck (5). This technique is described below and was used in isolating immunoglobulin-like molecules from bursectomized, irradiated chicken serum.

Solid immune adsorption

Isoliation of new immunoglobulin-like molecules was performed by immune adsorption as described by Avrameas and Ternynck (5). 10 ml of rabbit anti-IgM was polymerized by slowly adding 3-4 ml of 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.0). After 3 hr of polymerization at room temperature, the product was washed with 0.2 M phosphate buffer (pH 7.2), 0.2 M glycine·HCl buffer (pH 2.8), and phosphate-buffered saline (PBS, pH 7.6).

10 ml of agammaglobulinemic chicken serum was mixed with polymerized anti-IgM antiserum and stirred at room temperature for 2 hr. After immune adsorption, the polymer was exhaustively washed with PBS by centrifugation until the supernatant exhibited an A280 below 0.05. The adsorbed protein was then eluted by treating the polymer–protein complex with 0.2 M glycine·HCl buffer (pH 2.8) two to three times. Glycine·HCl buffer was removed from the protein solution by dialysis at 4°C against PBS, and the protein was concentrated by lyophilization and subjected to sodium dodecyl sulfate (SDS)–acylamide gel electrophoresis.

Electrophoresis at pH 7.2 was performed in 5.0% acrylamide, 0.5 M urea, and 0.1% SDS as described previously (6, 7). Lyophilized protein or serum was dissolved in solution I (10 M urea, 0.5 M Tris·HCl buffer (pH 8.5), and 1% SDS) to contain 1–2 mg/ml of protein and dialyzed overnight against 0.1 M sodium phosphate buffer (pH 7.2) containing 0.1% SDS. 50–100 μl of the protein solution was applied to the gel (0.6 × 6 cm) and electrophoresis was performed at 8 mA/gel for 3.5 hr.

Reduction and alkylation of immunoglobulins was performed in solution I with 0.23 M β-mercaptoethanol and 0.27 M iodoacetamide.

Abbreviations: PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate.
Serum protein, which precipitates with anti-IgM, was first discovered by immunoelectrophoresis in bursectomized--irradiated chickens (Fig. 1). This protein was shown not to contain light chains (k or λ) because it did not precipitate with anti-IgG (Fig. 1a) or anti-light chains. It is precipitated, however, either by anti-IgM (Fig. 1b) or by anti-μ (Fig. 1c). In contrast to IgM of normal chicken serum, which makes a long diffuse band, this new protein revealed a short arc around the well (Fig. 1b, upper arch) reflecting a more homogeneous character of possibly monoclonal origin. In immunoelectrophoresis against anti-normal chicken serum, bursectomized chicken serum revealed the same distinct band around the well (Fig. 1d, upper pattern), whereas such a band was not found in normal chicken serum (Fig. 1d, bottom pattern). This result indicates that this protein, which reacts to anti-IgM, may be a unique protein present in agammaglobulinemic chickens, and that it is different in structure from classical IgM and IgG. In other words, this protein may be present only in bursectomized chicken, and normal chicken may have it in such small quantities that it cannot be detected by immunoelectrophoresis. A similar immunoelectrophoretic pattern was published previously (1); this particular immunoglobulin-like entity, however, was not recognized.

Further investigation of the structure of this protein were attempted by means of SDS--acylamide gel electrophoresis. Bursectomized and normal chicken serum, 10 μl each, were analyzed in SDS--acylamide gel (Fig. 2). In contrast to normal chicken serum (Fig. 2b), which shows IgM and IgG bands, bursectomized chicken serum had a slow migrating band instead of IgM or IgG (Fig. 2b). This band could not be clearly demonstrated in normal chicken serum; its molecular weight appears to be about 300,000 as estimated by relative migration from the origin (Fig. 3). Therefore, the molecular size of this immunoglobulin-like molecule is smaller than IgM, which in the chicken has a molecular weight of 900,000 (8). This estimation of molecular weight was also confirmed by Sephadex G-200 column chromatography (Fig. 4). When

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**RESULTS**

**Microimmunoelectrophoresis**

Microimmunoelectrophoresis was performed by the method of Scheidegger (12).

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**Fig. 1.** Immunoelectrophoresis of bursectomized--irradiated chicken serum (top well) and normal chicken serum (bottom well), against (a) Anti-IgG, (b) Anti-IgM, (c) Anti-μ, (d) Anti-chicken serum.

The gels were fixed and stained by 1% amido black in 8% acetic acid for 3 hr and destained by 8% acetic acid.

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**Fig. 2.** Schematic representation of stained 5% polyacrylamide electrophoresis disc gels showing the relative migrations of the polypeptide chains. (a) Bursectomized--irradiated-chicken serum, (b) normal chicken serum, (c) IgM, (d) IgG.

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**Fig. 3.** Molecular weight determination from SDS--acylamide gel electrophoresis of (a) immunoglobulin-like molecules in serum and (b) immunoglobulin-like molecule isolated by immune adsorption. Standards are: (1) E. coli β-galactosidase (EC 3.2.1.23), (2) chicken albumin, (3) porcine pepsin, (4) trypsin, (5) cytochrome c.
agammaglobulinemic chicken serum, 3 ml, was filtered at room temperature through a Sephadex G-200 column (100 X 2.5 cm), the protein reacting with anti-IgM was found in the exclusion volume, as was IgM. The structural distinction of this protein from IgM was further confirmed by studying the isolated protein from bursectomized chicken serum. Since this protein was precipitated by specific anti-IgM, it was isolated by the solid immune adsorption method as described. The protein eluted from polymerized anti-IgM was lyophilized, dissolved in solution I, and subjected to SDS-acrylamide gel electrophoresis. It showed two major bands, one much denser and more slowly migrating than the other (Fig. 5c). The denser band had a molecular weight slightly larger than IgG (approximately 190,000), which was simultaneously run as control marker. The second, less distinct band corresponds to the size of chicken IgG heavy chain (γ-chain, molecular weight approximately 70,000, ref. 8). Both bands combined may have a molecular weight of approximately 300,000. This protein appears to be acid-labile because it was partially broken down by treatment with glycine-HCl buffer during the isolation procedure.

When the protein was reduced and alkylated, as was IgG (Fig. 5b), no band that corresponds to light chains was detected (Fig. 5d). This result indicates that this immunoglobulin-like molecule consists mainly of μ-chains and agrees with the findings by immunoelectrophoresis that failed to reveal light chain determinants with specific anti-IgG and anti-light chain antisera.

DISCUSSION

A new class of immunoglobulin is described, from studies of in ovo bursectomized and irradiated chickens, that did not have IgG and IgM. This conclusion is derived from the experimental results summarized below: (a) the new immunoglobulin has a molecular weight of 300,000, and is easily degraded into smaller subunits by acid treatment; (b) it consists mainly of heavy chains, which crossreact serologically with anti-μ; (c) it has no light chains, as shown by SDS-acrylamide gel electrophoresis after reduction and alkylation, and by immuno-electrophoresis.

During development of the lymphoid system in the chick embryo, it was shown that synthesis of IgM always precedes that of IgG (3). It was further shown that, within the bursa of Fabricius, IgG-producing cells arise from a clone of IgM-producing cells (9). In such chickens, we describe an immunoglobulin-like molecule, which is made of μ-chains but has no light chains. Since the presence of the bursa is required for subsequent development of IgM and IgG, we think that this immunoglobulin-like protein may be a primordial immunoglobulin, which is synthesized either by lymphoid cells independent of the bursa or by lymphoid cells developing in the bursa before immunocompetent cells are differentiated to produce IgM. It seems to us of great importance to define the site of initial synthesis of this protein. It is unlikely that this protein is a product of degradation of other immunoglobulins because it continues to be present in serum of agammaglobulinemic chicken for as long as 4–5 months after hatching. Maternally derived immunoglobulins apparently have disappeared 4–6 weeks after hatching.

An immunoglobulin of similar structure was previously reported in lamprey from phylogenetic studies of immunoglobulins (10, 11). Lamprey immunoglobulin has a molecular weight of 320,000, and is mostly made of heavy chains without any light chains. The striking structural similarities of the primordial immunoglobulins reported in this paper to lamprey immunoglobulin is most provocative. Lamprey is a cyclostome and one of the most primitive vertebrates to exhibit immune responses. Immunoglobulins of lamprey have characteristics of molecular size and subunit structure very similar to the possible progenitor of the immunoglobulins that we found in agammaglobulinemic chickens. Such an analogy between these immunoglobulins strengthens the view that the primordial immunoglobulin reported here is a primitive molecular form, representing a key to understanding in molec-
ular terms both the ontogenetic and phylogenetic development of the lymphoid system.

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