An ancestor of avian IgY was the evolutionary precursor of mammalian IgG and IgE, and present day chicken IgY performs the function of human IgG despite having the domain structure of human IgE. The kinetics of IgY binding to its receptor on a chicken monocyte cell line, MQ-NCSU, were measured, the first time that the binding of a non-mammalian antibody to a non-mammalian cell has been investigated ($k_{+1} = 1.14 \pm 0.46 \times 10^5 \text{mol}^{-1}\text{sec}^{-1}$, $k_{-1} = 2.30 \pm 0.14 \times 10^{-3} \text{sec}^{-1}$, and $K_a = 4.95 \times 10^7 \text{m}^{-1}$). This is a lower affinity than that recorded for mammalian IgE-high affinity receptor interactions ($K_a \sim 10^{10} \text{m}^{-1}$) but is within the range of mammalian IgG-high affinity receptor interactions (human: $K_a \sim 10^8–10^9 \text{m}^{-1}$, mouse: $K_a \sim 10^{-2}–10^{-3} \text{m}^{-1}$). IgE has an extra pair of immunoglobulin domains when compared with IgG. Their presence reduces the dissociation rate of IgE from its receptor 20-fold, thus contributing to the high affinity of IgE. To assess the effect of the equivalent domains on the kinetics of IgY binding, IgY-Fc fragments with and without this domain were cloned and expressed in mammalian cells. In contrast to IgE, their presence in IgY has little effect on the association rate and no effect on dissociation. Whatever the function of this extra domain pair in avian IgY, it has persisted for at least 310 million years and has been co-opted in mammalian IgE to generate a uniquely slow dissociation rate and high affinity.

IgY is the principal serum antibody of amphibians, reptiles, and birds and shares a common ancestor with both mammalian IgG and IgE (1, 2). The cDNA sequence of the chicken upsilon (υ) heavy chain (3) reveals that, like more basal amphibian IgY (4, 5), avian IgY contains a domain pair (Cυ2)4 that has been conserved in mammalian IgE (as Ce2) but truncated to form the “hinge” region in mammalian IgG (3). Thus, an orthologous domain pair must have existed in the common (IgY-like) ancestor prior to its duplication and subsequent divergence in the mammalian lineage. Avian IgY is the closest extant protein to this ancestor and therefore the most logical choice for study of the evolution of modern IgG and IgE from an ancient IgY-like ancestor.

IgG, the principal mammalian serum antibody, aggregates and facilitates opsonization of antigens, activates complement, and provides protection for the fetus following transport across the placenta. IgE does not activate complement nor cross the placenta but can sensitize effector cells (principally mast cells and basophils) and typically mediates anaphylactic reactions (6). It is well known for its involvement in allergic disease but probably also provides defense against parasitic infections (7).

Avian IgY appears to combine the functions of mammalian IgG and IgE. As the major serum antibody, it provides defense against infection (8) but also mediates anaphylaxis (9). Chicken mast cells activated by IgY are responsible for local anaphylactic reactions in the gut, which play a role in defense against protozoan infections (10, 11). Antibody-dependent hypersensitivity and fatal systemic anaphylactic shock have also been demonstrated in chickens (9, 12, 13) and shown to be mediated by basophils (14), which are much more numerous than mast cells in birds (in contrast to mammals in which the reverse is true) (15). These phenomena constitute indirect evidence for the presence of IgY receptors on both chicken monocytes/macrophages and basophils.

The υ heavy chain of IgY consists of four constant Ig domains and one variable Ig domain (3), as in mammalian IgE. It is clear from sequence comparisons that after the divergence of the synapsids 310 million years ago (16, 17), but before the divergence of the non-eutherian mammals 166 million years ago (18), duplication of the υ-gene occurred; this was followed by differentiation into the two Ig classes unique to mammals, IgG and IgE, as indicated by their presence in monotremes (19, 20). In IgG, the second domain of the heavy chain constant region condensed to form the hinge polypeptide (3), whereas in IgE, the domain was retained as Ce2 so that, like IgY, the Fc fragment consists of three constant Ig domain pairs. The two inter-heavy-chain disulfide bridges in the Ce2 domain are present also in Cυ2 at the same locations and in the hinge of IgG (Fig. 1).

Human mast cells and basophils express the high affinity IgE receptor (FcεRI), which has an affinity for IgE that is uniquely high: $K_a \sim 10^{10} \text{m}^{-1}$ (7). We have shown, through our studies receptor identified by Viertlboeck et al. (49); LRC, leukocyte receptor complex; Mb, megabase; my, million years; FACS, flow cytometry; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

**Avian IgY Binds to a Monocyte Receptor with IgG-like Kinetics Despite an IgE-like Structure**

Received for publication, February 19, 2008, and in revised form, March 17, 2008 Published, JBC Papers in Press, April 9, 2008, DOI 10.1074/jbc.M801321200

**Alexander I. Taylor**1,2, Hannah J. Gould, Brian J. Sutton, and Rosaleen A. Calvert2,3

From the Randall Division of Cell and Molecular Biophysics, King’s College London, New Hunt’s House, Guy’s Campus, London SE1 1UL, United Kingdom

The abbreviations used are: Cυ2, Cυ3 second and third constant domains of IgY; Ce2, Ce3, second and third constant domains of IgE; IgE-Fc, Fc fragment of IgE; IgY-Fc, Fc fragment of IgY; Fab, antigen-binding fragment; Fcυ2–4, Fcυ-Fc fragment containing heavy chain constant domains 2, 3, and 4; Fcε2–4, IgE-Fc fragment containing heavy chain constant domains 2, 3, and 4; Fcε3–4, IgY-Fc fragment containing heavy chain constant domains 3 and 4; CH, constant heavy chain domain; FcεRI, α chain of the high affinity receptor for IgE; CD23, low affinity receptor for IgE; FcγRI, high affinity receptor for IgG; FcεRI, high affinity receptor for IgA; CHIR-AB1, IgY

---

1 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 Supported by a Medical Research Council (UK) studentship.

3 Supported by the Biotechnology and Biological Sciences Research Council (UK).

4 To whom correspondence should be addressed. Fax: 44-207-848-6435; E-mail: rosy.calvert@kcl.ac.uk.
with IgE, IgE-Fc, and a subfragment of IgE-Fc consisting of only the Ce3 and Ce4 domains termed Fcε3-4 (21), that the high affinity is due to the slow dissociation rate from FcεRI (22, 23) and that the Ce2 domains are in part responsible (24). With a half-life on tissue mast cells of 2–3 weeks (25), IgE can effectively and persistently sensitize these cells for rapid degranulation upon encounter with antigen (allergen). The rapidity of degranulation is responsible for the life-threatening response of anaphylaxis, making an understanding of the mechanism by which the Ce2 domains alter the dissociation rate of IgE from its receptor a medically important issue.

Ce2-like domain pairs have persisted since their first appearance in a common ancestor that unites all terrestrial vertebrates. To assess their effect on the antibody-Fc receptor interaction, we have expressed two avian IgY-Fc fragments that differ only by the presence (Fcε2–4) or absence (Fcε3–4) of the Ce2 domain pair and measured their binding to the chicken monocyte cell line, MQ-NCSU, as well as that of whole IgY. This is the first report of the cell binding properties of a non-mammalian antibody and also addresses the question of whether the “extra” Ce2 domain pair in IgY necessarily leads to a high affinity interaction.

**EXPERIMENTAL PROCEDURES**

**Cloning of Fcε2–4 and Fcε3–4**—There is no intron between the CH1 and CH2 exons in chicken (26). Hence, the N terminus of chicken Ce2, Val-231 by the numbering system of Suzuki and Lee (27), was identified by a Clustal W alignment of the sequences of the heavy chain constant region of human IgE (28) and of duck IgY (29) with that of chicken IgY taken from Parvari et al. (3). The second residue of Ce2 is a cysteine that in IgE forms an intra-chain disulfide bond with Ce1; it was therefore mutated to an alanine in Fcε2–4, and has similarly been mutated in Fcε2–4. The N-terminal 4 amino acids of IgY-Fc were Asp-Ile (from an EcoRV site) Val-Ala.

Fce3–4 included the residues Asp-Ile from an EcoRV restriction site and 2 amino acids from Ce2, one of which, a cysteine, was thought to be required to form a functionally important inter-chain disulfide bond (21). For this reason, a similar Fcε3–4 with an N terminus including Cys-340 was cloned and expressed, but the protein formed intra-chain disulfide bonds with the extra Cys-347 in chicken IgY (Fig. 1). A C347S mutant led to expression of a functional protein, Fcε3–4, which has been used for the study reported here.

The polymerase chain reaction was used to engineer EcoRV and PmeI sites onto the 5’ and 3’ termini, respectively, of both Fcε2–4 and Fcε3–4 cDNA from a chicken spleenocyte library (kindly donated by Dr. John Young, Institute of Animal Health, Compton UK). Using a holding vector, a PmeI site was introduced after the stop codon of IgE-Fc so that using EcoRV and PmeI, IgY-Fc could be exchanged for IgE-Fc and then inserted into the pRY vector as a HindIII-HindIII cassette (30). Vectors were transfected into NS0 mouse myeloma cells for production of stable clones expressing Fcε2–4 or Fcε3–4.

**Purification and Characterization of IgY-Fc Fragment**—NS0 mouse myeloma cell supernatants were purified by elution from an anti-IgY-agarose affinity column (Gallus Immunotech, DAIgY-AGA) with 0.2 M glycine, pH 2.5, and dialyzed against phosphate-buffered saline (PBS) containing 0.05% sodium azide. Protein concentrations were measured in a Cary 50 spectrophotometer (Varian). The molar extinction coefficients for IgY, Fcε2–4, and Fcε3–4 were calculated using ProtParam software (31) and were 209,125, 90,965, and 62,505 mol⁻¹ cm⁻¹, respectively. Proteins were analyzed on denaturing SDS gels containing 12% acrylamide and the buffer system of Laemmli (32). Western blots used a semidry transfer method (33), and anti-IgY horseradish peroxidase (Promega, G135A) was used for detection.

**Cell Lines**—The MQ-NCSU cell line was provided by Dr. M. A. Qureshi, National Program Leader, Animal Genetics, United States Department of Agriculture Cooperative State Research Education and Extension Service, Washington, D.C. (34). Cells were grown at 37 °C in modified LM-Hahn medium omitting fetal bovine serum and chick serum, and thereby exogenous IgY, but including 8% ultra-low IgG fetal bovine serum (Invitrogen, catalog number 16250-078).

**Flow Cytometry (FACS)**—FACS was used to detect immunostained cells following their incubation with specific antibodies and/or antibody conjugates. In all experiments, cells were washed (by centrifugation at 1000 × g for 5 min) and incubated

---

**FIGURE 1.** The domain structure of avian IgY and its mammalian homologues, IgG and IgE. Schematic representations of the antibodies composed of variable (V) or constant (C) immunoglobulin domains that make up the heavy (H or γεκ/) and light (L) chains are shown with Fab and Fc fragments circled. Inter-chain disulfide bonds are shown as solid (known) or dashed (putative) lines. The domain structure of IgG shown here is that of human IgG1, with the hinge region represented by a curved line linking Cγ1 and Cγ2. Other subclasses of IgG vary in hinge length and number of inter-heavy chain disulfides.

---

**JUNE 13, 2008 • VOLUME 283 • NUMBER 24**
**IgY Binding to Cells**

FIGURE 2. *IgY binding to chicken monocytes is dependent on the Fc region.* Prior to detection with mouse anti-C(2) (followed by anti-mouse IgS-fluorescein isothiocyanate (Igs-FITC)), cells (shaded histograms) were incubated in PBS-BSA containing 20 μM IgY-Fab alone (A), 50 nM IgY alone (B), or 50 nM IgY + 20 μM IgY-Fab (C). Control cells (open histograms) were incubated with PBS-BSA alone before detection. Values shown are ratios of mean fluorescence intensity of each sample to that of control cells.

FIGURE 3. **Characterization of IgY and IgY-Fc fragments.** Whole IgY from chicken serum and recombinant IgY-Fc fragments secreted from mouse myeloma (NS0) cells were purified by affinity chromatography and analyzed using denaturing (SDS) 12% polyacrylamide gels, either stained with Coomassie Brilliant Blue (A and B) or Western-blotted with polyclonal anti-IgY antibodies (C and D). Samples were either non-reduced (A and C) or reduced with 2% β-mercaptoethanol (B and D). Lane 1, IgY. Lane 2, Fc2–4. Lane 3, Fc3–4 (C3405). Molecular weight markers are shown in kDa.

for 10 min in PBS containing 1% bovine serum albumin (BSA) (Sigma, catalog number A7030) before staining for 1 h at 4 °C with chicken IgY-(Fab)2 (Rockland, catalog number 003-0104), (Fab)2 + whole IgY, IgY, or buffer before incubation with a mouse monoclonal antibody (Sigma, catalog number C-7295), which was found to be an anti-C(2),5 and detection by antimouse fluorophore-conjugated secondary antibodies (Dako, catalog number F0479). Cells were washed twice with PBS-BSA both before and after all antibody incubation steps and fixed in PBS + 4% formaldehyde (Sigma, catalog number F1635) before analysis in a FACSCalibur instrument (BD Biosciences).

**Radioiodination of Antibodies and Antibody Fragments—Affinity-purified IgY (Stratech, catalog number 003-000-003) and IgY-Fc were radiolabeled with 125I (PerkinElmer Life Sciences, catalog number NEZ033A001MC) using the chloramine-T method (35).** Desalting spin columns (Pierce, catalog number 89849) were used to remove unincorporated 125I and exchange the radiolabeled IgY or IgY-Fc into PBS, 0.05% sodium azide following the manufacturer’s protocol. The specific activity in cpm/μg of each radioligand was determined by estimation of the protein concentration (by UV spectrophotometry) and cpm of radioactivity, using a Wizard 1480 γ counter (PerkinElmer Life Sciences).

**Cell Binding Assays—MQ-NCSU cells** were washed in PBS, 1% BSA, 0.05% sodium azide, pH 7.4, and incubated at 23 °C for 10 min before resuspension at 107 cells/ml. In experiments to determine the association rate constant, duplicate samples of 106 cells were incubated with 10 nM 125I-labeled IgY, Fc2–4, or Fc3–4 at 23 °C. At regular intervals, cells were centrifuged through 200 μl of phthalate oil (36), and the radioactivity of the cell pellet was determined using a Wizard 1480 gamma counter (PerkinElmer Life Sciences). The initial rate of association was determined from a linear fit of cpm *versus* time. Standard errors were generated by the fitting procedure but, due to uncertainty in number of the receptors per cell (see “Discussion”), larger errors have been added to the final values.

Dissociation experiments were performed using the method of Kulczycki and Metzger (37) but with all steps carried out at room temperature; cells were first incubated with 10 nM 125I-labeled IgY, Fc2–4, or Fc3–4 for 1 h and then resuspended in an excess of unlabeled IgY, and their radioactivity was assayed as above. Nonspecific binding was measured by incubating cells with an excess (350 nM) of unlabeled IgY for 1 h at room temperature before the addition of 10 nM labeled IgY, and the γ counts obtained were subtracted from the total bound counts. The mean number of receptor sites per MQ-NCSU cell was determined by titrating to saturation with 125I-labeled IgY or Fc2–4 of known specific activity. Dissociation rate constants were determined both by non-linear regression analysis from a plot of cpm *versus* time (according to Equation 20, p88, in Goodrich and Kugel (38)) and from linear regression analysis of a plot of ln(cpm) *versus* time.

**RESULTS**

The much higher affinity of IgE for its receptor on mast cells when compared with IgG for any of its receptors and the role of the extra Cε2 domain pair in IgE prompted this study of chicken IgY, the closest extant immunoglobulin to the ancestor that evolved into all three antibodies.

In a preliminary FACS experiment, it was established that chicken serum IgY binds to MQ-NCSU cells in an Fc-dependent manner; IgY Fabs at 400× molar excess over whole IgY were unable to bind to cells or to inhibit IgY binding (Fig. 2). The binding constants determined in the studies described below are thus for IgY and IgY-Fc binding to an IgY-Fc receptor on a chicken monocyte cell line.

The action of papain on IgY does not give rise to an Fc2–4 fragment since the Cε2 domain is digested away (27). Hence, to assess the role of Cε2, Fc2–4 and Fc3–4 were cloned and expressed as recombinant proteins. Purified IgY (Fig. 3, lane 1),

---

5 A. I. Taylor, H. J. Gould, B. J. Sutton, and R. A. Calvert, unpublished observations.
minor band of FcIgY of approximately the expected molecular mass with only a
deviation or heterogeneity of glycosylation. Western blots of the same
bonds on exposure to 100 °C in SDS buffer (39) and not to degra-
d reduction of the small amount of heterogeneous-sized material in unre-
ciated as shown by periodic acid Schiff staining,5 and all had
chain was made. Measurements on two different days led to an estimate of 20,000, and the
likely error in this value is discussed below. The value obtained for \(k_{-1}\) is
0.04 \times 10^5 \text{ mol}^{-1} \text{ s}^{-1} \) (Fig.
C). The two rate constants lead to a
value 3.5 \times 10^5 \text{ mol}^{-1} \text{ s}^{-1}, and for Fc3-4, it was 1.64 \pm 0.01 \times 10^6 \text{ mol}^{-1} \text{ s}^{-1},
which differ only by a factor of about 2 (Fig. 5C). This is not a
significant difference given the errors inherent in this tech-
nique (discussed below).

**DISCUSSION**

These studies determine for the first time the binding kinet-
ics of IgY and its Fc fragments to cells and demonstrate that the
affinity is more similar to that of IgG for its high affinity recep-
tor than IgE for its high affinity receptor. Furthermore, the
kinetics of the interaction are not affected by the presence or
absence of the Cu2 domain pair, in contrast to the effect that the
Ce2 pair has on the kinetics of IgE binding to FcεRI (24).

IgY dissociates from its receptor on the MQ-NCSU chicken
monocyte cell line with a rate constant of \(k_{-1} = 2.30 \pm 0.14 \times 10^{-3} \text{ s}^{-1}\) at 23 °C (Fig. 4A). Although 10 times faster than the
dissociation of human IgG1 from the monocyte cell line U937
\((k_{-1} = 1.1 \pm 0.1 \times 10^{-3} \text{ s}^{-1})\) (41), \(k_{-1}\) for IgY is close to the
value 3.5 \times 10^{-3} \text{ s}^{-1} (no error reported) measured for the dis-
sociation of mouse Ig subclass 2a from the mouse macro-
phage cell line P388D (42). Dissociation of human Ig from
(cord blood) mast cells is so slow by comparison that even at
37 °C, values of 6.05 \times 10^{-5} \text{ s}^{-1} (43) (no error given) and 6.93 \pm
0.54 \times 10^{-5} \text{ s}^{-1} (44) have been recorded.

A value of 1.14 \pm 0.04 \times 10^{9} \text{ mol}^{-1} \text{ s}^{-1} has been determined for the association rate constant of IgY with MQ-NCSU cells,
although a much larger error is inherent in this value (and con-
sequently the \(K_a\)) than in that of the dissociation rate constant.
The number of receptors per cell is a factor in the calculation,
and it has been reported that Fc receptor number on human
monocytes may vary on average by \pm 25% and up to 40% (45);
the error in \(k_{+1}\) for IgY may therefore be as much as \pm 40%. The
value of \(k_{+1}\) for IgE binding to the FcεRI α chain expressed on
Chinese hamster ovary cells at 25 °C is 3.1 \pm 0.3 \times 10^{5} \text{ mol}^{-1} \text{ s}^{-1} (30), and for human IgG1 to U937 cells, it is 2.1 \pm 0.2 \times 10^{5}
\text{ mol}^{-1} \text{ s}^{-1} (41). Thus, the association rate constant for human
IgE differs from the value determined here for IgY binding to

**FIGURE 4. Binding of IgY to chicken monocytes.** Dissociation experiments were performed by incubating
MQ-NCSU cells with 10 nm [125I]-IgY from chicken serum for 1 h followed by the addition of an excess of unla-
beled IgY. Cells were spun through phthalate oil, and their radioactivity (in cpm) was measured at regular
intervals and normalized to the initial value. The dissociation rate constant was determined by both non-linear
(A) and linear (B) regression analyses. Data from a single dissociation experiment are shown. Association
experiments were performed by measuring the radioactivity of cell pellets at regular intervals starting from the
addition of 10 nm [125I]-IgY. Mean data from two duplicate experiments are shown \pm S.E. (error bars) (C). All
experiments were performed at room temperature.

**FIGURE 5. Binding of IgY-Fc fragments to chicken monocytes.** Dissociation experiments were performed by incubating MQ-NCSU cells with 10 nm [125I]-labeled IgY-Fc2-4 or IgY-Fc3-4 for 1 h followed by the addition of an excess of unlabeled whole IgY. Cells were spun through phthalate oil, and their radioactivity (in cpm) was measured at regular
intervals and normalized to the initial value. The dissociation rate constant was determined, Fc3-4 (Fig. 5A), showing that the Cu2 domain pair does not have a detectable effect on the rate of dissociation. The association rate constant for Fc2-4 was 3.66 \pm 0.16 \times 10^5 \text{ mol}^{-1} \text{ s}^{-1}, and for Fc3-4, it was 1.64 \pm 0.01 \times 10^6 \text{ mol}^{-1} \text{ s}^{-1},
which differ only by a factor of about 2 (Fig. 5B). This is not a
significant difference given the errors inherent in this tech-
nique (discussed below).
MQ-NCSU cells by a factor of only 2.7, in contrast to the difference in affinity, which is about 700-fold ($K_a = 4.95 \times 10^7$ M$^{-1}$ for IgY; $K_a = 3.4 \times 10^{10}$ M$^{-1}$ for IgE) (30). The difference between IgY and IgE in their affinity for cells is therefore due almost entirely to a difference in their rates of dissociation (as it is for IgG when compared with IgE). The affinity of IgY reported here is within the range of mammalian IgG–high affinity receptor interactions: e.g. human $K_a \sim 10^8$–$10^9$ M$^{-1}$; mouse $K_a \sim 10^7$–$10^8$ M$^{-1}$ (46).

The presence of the Cε2 domain pair slows the rate of dissociation of IgE from FcεRI by a factor of approximately 20, as shown by a comparison of the binding kinetics of two IgE fragments, one with this domain (Fε2–4) and one without (Fε3–4) (22, 24). The influence of the Cε2 domains on the kinetics of IgY binding to cells was therefore investigated using two corresponding IgY fragments, Fε2–4 and Fε3–4, differing only by the Cε2 domain pair. The dissociation curves of Fε2–4 and Fε3–4 can be superimposed (Fig. 5A), showing that unlike IgE–Fc (24), the presence of the Cε2 domain pair has no effect on the rate of dissociation. Although the values for the association rate constants differ by a factor of 2.2 ($k_{-1} = 3.66 \pm 0.16 \times 10^7$ mol$^{-1}$ s$^{-1}$ for Fε2–4; $k_{+1} = 1.64 \pm 0.01 \times 10^5$ mol$^{-1}$ s$^{-1}$ for Fε3–4), the potential error in these values due to possible variability in cell receptor number of $\pm 40\%$ means that these values are not significantly different.

Although the Cε2 domains of chicken IgY do not appear to contribute to receptor binding on monocytes, this does not preclude a role in, for instance, binding to mast cells or basophils. However, cutaneous anaphylaxis experiments in chickens (9) have shown that IgY is not retained in tissue for as long as mammalian IgE (i.e. only for days rather than weeks), which suggests that the dissociation rate of IgY from mast cells is also more akin to that of IgG than IgE. A function for Cε2 is likely, however, as it has persisted in both mammals and birds for the 310 million years since Synapsida and Reptilia diverged, and it has recently been estimated that DNA coding for a non-functional protein domain has a half-life of only 4 million years in the genome (47, 48).

The association constant determined in the experiments described in this report ($K_a = 4.95 \times 10^7$ M$^{-1}$) is close to that determined for the interaction between chicken serum IgY and an extracellular domain of a member of the chicken leukocyte receptor complex (LRC), CHIR-AB1, as measured by surface plasmon resonance: $K_a = 3.70 \times 10^7$ M$^{-1}$ (49). Since CHIR-AB1 mRNA was detectable in chicken M11 primary macrophages (49), it is likely that CHIR-AB1 is the molecule responsible for IgY binding to MQ-NCSU monocytes in the data presented here. One caveat in comparing these two values is that association constants obtained by cell binding and SPR sometimes differ; $K_a$ for IgE binding to the FcεRIα chain displayed on Chinese hamster ovary cells is 10× higher than the value obtained by surface plasmon resonance (22, 24, 30). Furthermore, additional polypeptide chains in the native, cell-bound receptor may influence affinity.

Another issue to consider is whether the MQ-NCSU cells express more than one receptor. The human monocyte cell line, U937, expresses a number of both IgG and IgE receptors, and MQ-NCSU cells may express receptors homologous to these, to which IgY may in principle bind. However, in studies of IgG binding to U937 cells, only one receptor, FcyRII, is detected, despite the presence of others, FcyRII and FcyRIII, because the dissociation rates are too fast to be measured (50). Similarly, IgE binding to U937 cells detects neither CD23 (low affinity receptor for IgE) unless induced (because it is expressed at such a low level) (51) nor FcεRI (which requires neuraminidase treatment to unmask it) (52). The kinetic data reported here for IgY binding to MQ-NCSU cells suggest that a single receptor is being detected.

Comparison of the association rate constants measured here in cell binding for IgY with those determined by surface plasmon resonance (49) also shows good agreement: $1.14 \times 10^5$ mol$^{-1}$ s$^{-1}$ and $2.4 \times 10^5$ mol$^{-1}$ s$^{-1}$, respectively. These authors also measured the binding kinetics for an IgY–Fc fragment produced by papain digestion, consisting of a dimer of the Cε3 and Cε4 domains (49). Their value of $k_{+1} = 3.9 \times 10^6$ s$^{-1}$ for this fragment is $24 \times$ faster than that reported here, $k_{+1} = 1.64 \pm 0.01 \times 10^5$ mol$^{-1}$ s$^{-1}$, for the recombinant Fcε3–4. However, we also generated an Fc fragment by papain digestion and found, by N-terminal sequencing (27), that a substantial proportion of the material consists of an incomplete Cε3 domain; this may explain the discrepancy between the two $k_{+1}$ values.

The studies of Viertlboeck et al. (49) and, we believe, the data reported here, concern the binding of an antibody to a receptor coded within the chicken LRC (on microchromosome 31). The mammalian LRC is a 1-megabase gene cluster on human chromosome 19, consisting of 45 genes, of which 30 encode Ig-like receptors that have mostly HLA class I molecules as ligands (53). In mammals, antibody Fc receptors are encoded mainly within the Fc receptor gene family (on human chromosome 1), but there are two examples of Fc receptors encoded within the mammalian LRC: the IgA receptor FcαRI (CD89) and bovine FcyRIIB (54). There is a significant difference between the way in which human FcαRI interacts with IgA and how the “classical” Fc receptors belonging to the FcR gene cluster interact with IgG and IgE. Crystal structures of these Fc-receptor complexes show that in both IgG and IgE, the binding site lies in a similar region at the N terminus of the homologous Cε2 and Cε3 domains, respectively, whereas IgA interacts with its receptor through a site close to the junction between Cε2 and Cε4 (55). (It is not yet known to which domains of IgG bovine FcyRIIB binds, but the interaction does involve domain 1 of the receptor, which is characteristic of LRC receptor interactions, in contrast to FcR, which involve domain 2.) The chicken LRC sequence is incomplete, but already 103 genes highly related to each other have been identified (56); currently, only CHIR-AB1 has a known ligand. As CHIR-AB1 is more closely related to FcαRI than the Fc receptors for IgG or IgE, chicken IgY may bind to its receptor in a similar manner to that in which human IgA binds to FcαRI. If so, and if the receptor binding site in IgY lies between Cε3 and Cε4 (and not at the N-terminal region of Cε3), this would be consistent with our observation that the presence of Cε2 plays no role in the binding kinetics.

Intriguingly, the FcR family in chicken appears to be represented by just a single gene (57, 58), and our preliminary experiments show that this receptor does not bind IgY. The mam-
malian FcR complex contains many receptor homologues whose ligands are not antibodies, and these receptors are thought to play a role in immunoregulation (59). These observations, and the data presented in this report, lead us to speculate that Fc receptor function migrated from the LRC to the FcR locus (which subsequently expanded) during the last 310 million years. The migration of Fc receptor function may have been a consequence of the need to shift the binding site in the antibody Fc from the Cα1/Cα2 interface in the IgY-like ancestor of IgG and IgE, due to competition from bacterial Fc-binding proteins. Such proteins are known to bind to the Cα2/Cα3 interface in IgA (60) and the Cγ2/Cγ3 interface in IgG (61, 62), thereby disabling the immune response by blocking FcR interactions. This selection pressure has led to co-evolution of IgA and its receptor (63); a receptor mutating too slowly to keep up with changing bacterial peptide sequences becomes redundant and may persist only as a pseudogene (as in rabbits) (63) or be eliminated altogether (as in mouse) (64). The IgY-like ancestor of IgG and IgE may have overcome this selection pressure by shifting the binding site to the N-terminal region of the domain that evolved into Cγ2 and Cε3. Whatever the reason for the persistence of the Cε2 domains in IgY and its ancestors, its presence in the ancestor of IgE served as a preadaptation for the evolution of an antibody domain (Cε2) that contributes to the uniquely slow dissociation rate and high affinity for receptor and the consequent advantages this confers for defense against parasites.

Acknowledgment—We thank the Nuclear Medicine Department of Guy’s and St. Thomas’s Hospital for the use of their PerkinElmer Life Sciences Wizard gamma counter.

REFERENCES

1. Flajnik, M. F. (2002) Nat. Rev. Immunol. 2, 688–698
2. Warr, G. W., Magor, K. E., and Higgins, D. A. (1995) Immunol. Today 16, 392–398
3. Parvari, R., Avivi, A., Lentner, F., Ziv, E., Tel-Or, S., Burstein, Y., and Schechter, I. (1988) EMBO J. 7, 2309–744
4. Amemiya, C. T., Haire, R. N., and Litman, G. W. (1989) Nucleic Acids Res. 17, 5388
5. Fellah, J. S., and Charlemagne, I. (1988) Mol. Immunol. 25, 1377–1386
6. Murphy, K. M., Travers, P., Walport, M., and Janeway, C. (2008) Janeway’s Immunobiology, pp. 566–567, Garland Science, London
7. Gould, H. J., Sutton, B. J., Beavil, A. J., Beavil, R. L., McCloskey, N., Coker, H. A., Fear, D., and Smurthwaite, L. (2003) Annu. Rev. Immunol. 21, 579–628
8. Qureshi, M. A., Heggen, C. L., and Hussain, I. (2000) Dev. Comp. Immunol. 24, 103–119
9. Faith, R. E., and Clem, L. W. (1973) Immunology 25, 151–164
10. Caldwell, C. C., Hornyk, S. C., Pendleton, E., Campbell, D., and Knowles, A. F. (2001) Arch. Biochem. Biophys. 387, 107–116
11. Rose, M. E., Ogilvie, B. M., and Bradley, J. W. (1980) Int. Arch. Allergy Appl. Immunol. 63, 21–29
12. Bellavia, A., Marino, V., Gallo, E., Peri, S. M., Bentivegna, C., Agresti, L., and Di Bona, M. (1992) Immunopharmacol. Immunotoxicol. 14, 233–250
13. Chand, N., and Eyre, P. (1976) Br. J. Pharmacol. 57, 399–408
14. Wilson, A. B., and Heller, E. D. (1976) Int. Arch. Allergy Appl. Immunol. 51, 68–79
15. Chand, N., and Eyre, P. (1978) Avian Dis. 22, 639–645
16. Hedges, S. B. (2002) Nat. Rev. Genet. 3, 838–849
17. Reisz, R. R., and Muller, J. (2004) Trends Genet 20, 237–241
18. Bininda-Emonds, O. R., Cardillo, M., Jones, K. E., MacPhee, R. D., Beck, R. M., Greynyer, R., Price, S. A., Vos, R. A., Gittleman, J. L., and Purvis, A. (2007) Nature 446, 507–512
19. Vernersson, M., Aveskogh, M., and Hellman, L. (2004) Dev. Comp. Immunol. 28, 61–75
20. Vernersson, M., Aveskogh, M., Munday, B., and Hellman, L. (2002) Eur. J. Immunol. 32, 1415–1415
21. Shi, J., Ghirlando, R., Beavil, R. L., Beavil, A. J., Keown, M. B., Young, R. J., Owens, R. J., Sutton, B. J., and Gould, H. J. (1997) Biochemistry 36, 2112–2122
22. Cook, J. P., Henry, A. J., McDonnell, J. M., Owens, R. J., Sutton, B. J., and Gould, H. J. (1997) Biochemistry 36, 15579–15588
23. Henry, A. J., Cook, J. P., McDonnell, J. M., Mackay, G. A., Shi, J., Sutton, B. J., and Gould, H. J. (1997) Biochemistry 36, 15568–15578
24. McDonnell, J. M., Calvert, R., Beavil, R. L., Beavil, A. J., Henry, A. J., Sutton, B. J., Gould, H. J., and Cowburn, D. (2001) Nat. Struct. Biol. 8, 437–441
25. Geha, R. S., Helm, B., and Gould, H. (1985) Nature 315, 577–578
26. Zhao, Y., Rabban, H., Shizimu, A., and Hammarsrom, L. (2000) Immunology 101, 348–353
27. Suzuki, N., and Lee, Y. C. (2004) Glycobiology 14, 329–332
28. Kenten, J. H., Molgaard, H. V., Houghton, M., Derbyshire, B. R., Viney, J., Bell, L. O., and Gould, H. J. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6661–6665
29. Magor, K. E., Warr, G. W., Middleton, D., Wilson, M. R., and Higgins, D. A. (1992) J. Immunol. 149, 2627–2633
30. Young, R. J., Owens, R. J., Mackay, G. A., Chan, C. M., Shi, J., Hide, M., Francis, D. M., Henry, A. J., Sutton, B. J., and Gould, H. J. (1995) Protein Eng 8, 193–199
31. Walker, J. M. (2005) The Proteomics Protocols Handbook, p. 571–607, Humana Press, Totowa, NJ
32. Laemmli, U. K. (1970) Nature 227, 680–685
33. Kyhse-Andersen, J. (1984) J. Biochem. Biophys. Methods 10, 203–209
34. Qureshi, M. A., Miller, L., Lillehoj, H. S., and Ficken, M. D. (1990) Vet. Immunol. Immunopathol. 26, 237–250
35. McConahey, P. J., and Dixon, F. J. (1980) Methods Enzymol. 79, 210–213
36. Segal, D. M., and Hurwitz, E. (1977) J. Immunol. 118, 1338–1347
37. Kulczycki, A., Jr., and Metzger, H. (1974) J. Exp. Med. 20, 1676–1695
38. Goodrich, J. A., and Kugel, J. F. (2007) Binding and Kinetics for Molecular Biologists, p. 8, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
39. Ikeyama, S. (1987) Mol. Immunol. 24, 231–237
40. Ellman, G. L. (1958) Arch. Biochem. Biophys. 74, 443–450
41. Shopes, B., Weetall, M., Holowka, D., and Baird, B. (1990) J. Immunol. 145, 3842–3848
42. Unkeless, J. C., and Eisen, H. N. (1975) J. Exp. Med. 142, 1520–1533
43. Ishizaka, T., Helm, B., Nakata, K., Kanda, T., and Ishizaka, T. (1983) J. Exp. Med. 157, 815–826
44. Gross, H. J., and Eisen, H. N. (1975) J. Exp. Med. 142, 1520–1533
45. Karagiannis, S. N., Bracher, M. G., Beavil, A. J., Hunt, J., McCloskey, N., Witt, C., Pontarotti, P., and Christiansen, F. T. (2002) Trends Immunol. 23, 81–88
IgY Binding to Cells

54. Zhang, G., Young, J. R., Tregaskes, C. A., Sopp, P., and Howard, C. J. (1995) J. Immunol. 155, 1534–1541
55. Woof, J. M., and Burton, D. R. (2004) Nat. Rev. Immunol. 4, 89–99
56. Laun, K., Coggill, P., Palmer, S., Sims, S., Ning, Z., Ragoussis, J., Volpi, E., Wilson, N., Beck, S., Ziegler, A., and Volz, A. (2006) PLoS Genet. 2, e73
57. Fayngerts, S. A., Najakshin, A. M., and Taranin, A. V. (2007) Immunogenetics 59, 493–506
58. Taylor, A. I., Gould, H. J., Sutton, B. J., and Calvert, R. A. (2007) Immunogenetics 59, 323–328
59. Davis, R. S. (2007) Annu. Rev. Immunol. 25, 525–560
60. Pleass, R. J., Areschoug, T., Lindahl, G., and Woof, J. M. (2001) J. Biol. Chem. 276, 8197–8204
61. Sauer-Eriksson, A. E., Kleywegt, G. J., Uhlen, M., and Jones, T. A. (1995) Structure (Lond.) 3, 265–278
62. Deisenhofer, J. (1981) Biochemistry 20, 2361–2370
63. Abi-Rached, L., Dorighi, K., Norman, P. J., Yawata, M., and Parham, P. (2007) J. Immunol. 178, 7943–7954
64. Maruoka, T., Nagata, T., and Kasahara, M. (2004) Immunogenetics 55, 712–716