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Immunology

Expression of 12 rabbit IgA C_α genes as chimeric rabbit–mouse IgA antibodies

(transfectoma/isotype/allotype/immunoglobulin genes/polymeric immunoglobulin receptor-mediated transcytosis)

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ABSTRACT **Serologic analysis of rabbit secretory IgA initially identified two subclasses of IgA, IgA-f and IgA-g. Recent molecular genetic studies have resulted in the identification and cloning of 13 genes encoding the constant region (C) of rabbit IgA heavy chains. Each of these 13 C_α genes, $C_\alpha 1$–$C_\alpha 13$, was subcloned into an expression vector containing the *VDJ* (V, variable; D, diversity; J, joining) gene of a dansyl (DNS)-binding hybridoma antibody. The α heavy-chain constructs were transfected into SP2/0 cells producing murine light chains with specificity for DNS. Of the 13 resulting transfectomas, 12 were shown by ELISA to secrete DNS-binding chimeric rabbit–mouse IgA molecules. By immunoblot analysis, the 12 IgA-producing transfectomas were shown to secrete α chains ranging in size from 60 to 72 kDa. These data suggest that rabbit IgA may be composed of as many as 12 IgA isotypes. This is in marked contrast to mouse and human, in which only 1 and 2 IgA isotypes, respectively, are found. Serologic analyses, using anti-IgA-f and anti-IgA-g alloantisera, revealed that 11 of the 12 transfectoma IgAs reacted with anti-IgA-f and not with anti-IgA-g antibodies and that one reacted with anti-IgA-g and not with anti-IgA-f antibodies. Each of the IgA-producing transfectomas was cocultured with a Madin–Darby canine kidney cell line expressing the rabbit polymeric immunoglobulin receptor, and the transcytosed IgA antibodies were analyzed by immunoblots to determine whether they associated with secretory component (SC) through covalent or noncovalent interactions. Each of the 11 IgA-f isotypes was shown to bind SC by a disulfide linkage, whereas the single IgA-g isotype appeared to bind SC through noncovalent interactions only.**

IgA is the predominant immunoglobulin isotype in the secretory fluids of the mucosae and is critical as a first line of humoral immunity against environmental pathogens. Given the large surface area of the mucosae, the total daily production of IgA far exceeds the production of all other immunoglobulin isotypes combined (1). Early studies of rabbit secretory IgA (sIgA) resulted in the serologic identification of two subclasses of IgA, IgA-f and IgA-g (2, 3). These subclasses were shown to differ in their heavy-chain allotypic specificities (4), their types of bonding with secretory component (SC) (5), and their susceptibility to proteolytic digestion (6–8). A more detailed serologic analysis of colostral IgA indicated that there were more than two subclasses of rabbit sIgA (9). Southern blot analysis of genomic DNA, in fact, indicated that the rabbit genome has a minimum of 10 C_α genes (C, constant region) (10). In more recent studies, 13 nonallelic germline C_α genes have been cloned from recombinant phage and cosmid libraries (ref. 11; R. C. Burnett and K.L.K., unpublished results).

We now demonstrate that 12 of the 13 germ-line C_α genes are expressible in an *in vitro* expression system that generates chimeric rabbit–mouse anti-dansyl (DNS) IgA antibodies comprised of mouse variable region heavy chain (V_H), rabbit C_α, and mouse κ chains. The 12 expressed chimeric IgA antibodies were characterized as to their isotypic specificities and binding properties to SC.

MATERIALS AND METHODS

Chimeric α Heavy-Chain Gene Constructs and Their *in Vitro* Expression. The eukaryotic expression vector pML-gpt/DNS-VDJ was generously provided by V. T. Oi (Becton Dickinson) (12) and contains a murine *VDJ* (D, diversity; J, joining) gene specific for the DNS hapten and the murine heavy-chain enhancer element. Each of 13 rabbit germ-line C_α genes (Table 1) was subcloned from a cosmid or phage clone (ref. 11; R. C. Burnett and K.L.K., unpublished results) into a *Sal* I site of the expression vector.

The $C_\alpha 13$ gene fragment was shown by nucleotide sequence analysis to be an incomplete gene (R. C. Burnett and K.L.K., unpublished data). The *Eco*RI site at the 5′ end of this fragment was shown to be in the codons encoding amino acid residues 19–21; thus, the $C_\alpha 13$ gene lacked the DNA encoding the N-terminal 19 amino acid residues of CH1. An *Eco*RI site was also present in the $C_\alpha 8$ gene in the codons encoding amino acid positions 19–21; thus, a chimeric $C_\alpha 8$–$C_\alpha 13$ gene was constructed, for expression, by splicing a 2.7-kilobase (kb) *Bgl* II/*Eco*RI fragment of the $C_\alpha 8$ gene, containing DNA encoding the N-terminal 19 amino acids plus ≈2.6 kb of 5′ flanking region, to the 5.5-kb *Eco*RI/*Hin*dIII fragment of the $C_\alpha 13$ gene. This construct resulted in a gene whereby the N-terminal 19 amino acids of the CH1 domain were encoded by the $C_\alpha 8$ gene and the remainder of the α chain was encoded by the $C_\alpha 13$ gene. All of the C_α genes were cloned from rabbit DNA of the *G* heavy-chain haplotype (IgA allotypes f71 and g75) except for $C_\alpha 5$ and $C_\alpha 6$, which were cloned from rabbit DNA of the *C* heavy-chain haplotype (IgA allotypes f72 and g74), and $C_\alpha 12$, which was cloned from rabbit DNA of the *J* heavy-chain haplotype (IgA allotypes f70 and g76) (13).

Murine SP2/0 myeloma cells were transfected by electroporation (14) with a plasmid, pSV184-neo/DNS-VCK, containing the κ-chain gene of a murine DNS-binding hybridoma antibody (kindly provided by V. T. Oi). Light-chain transfectants were selected for G418 (GIBCO) resistance (15), and a stable clone expressing the murine light chain, designated SVCK, was used for the electroporation-mediated transfec-

Abbreviations: C, constant; DNS, dansyl; SC, secretory component; TCF, transfectoma culture fluid; sIgA, secretory IgA.

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Table 1. Restriction fragments and heavy-chain haplotypes of germ-line C_α genes subcloned into the DNS–VDJ expression vector

Gene	Source (haplotype)*	Fragment used	5′/3′ sequence,† kb	S_α‡	Ref.
$C_\alpha 1$	Cos 28.7 (G)	12.0-kb *Bam*HI	0.8/9.8	−	11
$C_\alpha 2$	Cos 28.7 (G)	8.4-kb *Bam*HI	0.7/6.4	−	11
$C_\alpha 3$	Cos 24.1 (G)	6.4-kb *Bam*HI	4.0/1.0	+	11
$C_\alpha 4$	Ph 12.1 (G)	8.0-kb *Bam*HI	0.3/6.3	−	11
$C_\alpha 5$	Cos 21.1 (C)	9.2-kb *Bam*HI	6.9/0.9	+	11
$C_\alpha 6$	Cos 21.1 (C)	7.0-kb *Bam*HI	4.9/0.7	+	11
$C_\alpha 7$	Cos 13.2 (G)	2.8-kb *Bam*HI	0.1/1.3	−	11
$C_\alpha 8$	Ph 1.1 (G)	9.3-kb *Bgl* II	2.6/5.2	+	§
$C_\alpha 9$	Ph 1.1 (G)	6.5-kb *Bgl* II/ *Bam*HI	3.4/1.6	+	§
$C_\alpha 10$	Ph 160.1 (G)	8.0-kb *Bam*HI	1.9/4.7	−	§
$C_\alpha 11$	Ph 160.1 (G)	2.1-kb *Bam*HI	0.2/0.5	−	§
$C_\alpha 12$	Ph 13.1 (J)	8.1-kb *Eco*RI	5.0/1.8	+	§
$C_\alpha 13$	Ph 23a (G)	5.5-kb *Eco*RI/ *Hin*dIII¶	2.6/3.6	+	§

*Immunoglobulin heavy-chain haplotypes from which the cosmid (Cos) or phage (Ph) clones were derived: C, IgA allotypes f72 and g74; G, IgA allotypes f71 and g75; J, IgA allotypes f70 and g76.
†Kilobases of nucleotide sequence 5′ of C_α coding region contained in the C_α subclone/kilobases of nucleotide sequence 3′ of C_α coding region contained in the C_α subclone.
‡Presence (+) or absence (−) of switch sequences (S_α) in the C_α subclone.
§R. C. Burnett and K.L.K., unpublished data.
¶This gene was modified at the 5′ end as described in *Materials and Methods*. The 2.6 kb at the 5′ end of the chimeric $C_\alpha 8$–$C_\alpha 13$ gene is derived from the $C_\alpha 8$ gene.

tion of each of the α heavy-chain constructs. Transfectants were selected for mycophenolic acid (GIBCO) resistance (16), and individual transfectants were cloned by limiting dilution.

Antisera. Rabbit alloantisera, anti-f71 (W290), anti-f72 (L76-7), anti-g74 (K351-2), and anti-g75 (J256-2) were prepared as described (3, 17, 18). Anti-rabbit α chain, anti-rabbit γ chain, anti-rabbit SC, and anti-mouse immunoglobulin, all prepared in goats, were adsorbed by, and specifically purified on, immunoglobulin- and/or SC-containing Sepharose immunosorbent columns (19). The purified antibodies were tested for specificity in an ELISA by using immunoglobulin-coated microtiter plates (20). By these analyses, the anti-rabbit α-chain antibody did not react with rabbit or mouse IgG; anti-mouse immunoglobulin antibody did not react with rabbit IgG or sIgA; anti-rabbit γ-chain antibody did not react with rabbit IgA or mouse IgG; and anti-rabbit SC antibody did not react with rabbit α chains. Immunoblot analysis of proteins separated by $NaDodSO_4$/PAGE demonstrated that the anti-rabbit IgA antiserum (heavy- and light-chain specific) used for immunoprecipitation did not react with SC. Antibodies used for protein immunoblot analyses were ^{125}I-labeled (21) and antibodies used for ELISA were biotinylated (22).

Serologic Analysis of Chimeric IgA Antibodies by ELISA. Ninety-six-well microtiter plates (Falcon, Pro-bind) were coated with DNS-gelatin (50 μg/ml) (23) and ELISA was performed on the transfectoma culture fluids (TCFs) essentially as described (20). Biotinylated anti-rabbit α-chain antibody or biotinylated anti-mouse immunoglobulin antibody was used in conjunction with Vectastain, avidin–biotin complex (ABC) (Vector Laboratories). The IgA-f and IgA-g isotypes of the chimeric antibodies were detected with anti-IgA-f and anti-IgA-g alloantisera and biotinylated anti-rabbit γ-chain antibody. Absorbance was determined spectrophotometrically in an ELISA reader (Bio-Tek, Burlington, VT) at 405 nm. Binding of SC to the α13-g transfectoma IgA was performed on DNS-gelatin-coated ELISA plates with biotinylated anti-rabbit α-chain or anti-rabbit SC antibodies.

Immunoprecipitation of Chimeric IgA Antibodies and Protein Immunoblot Analysis. Transfectomas were cultured in serum-free medium (RPMI 1640) supplemented with 1% Nutridoma SP (Boehringer Mannheim). The TCFs or cell lysates were immunoprecipitated with anti-rabbit IgA antiserum and formalin-fixed *Staphylococcus aureus* (9). The immunoprecipitates were reduced with 2-mercaptoethanol (5% final concentration) and analyzed by immunoblots of $NaDodSO_4$/polyacrylamide gels. The gels were electroblotted onto nitrocellulose membranes (24) and blocked with 2% nonfat dry milk (Carnation); blots were developed with ^{125}I-labeled anti-rabbit α-chain antibody, ^{125}I-labeled anti-mouse immunoglobulin antibody, or a mixture of the two. Autoradiographs (Kodak XAR-5) were made of the blots.

***In Vitro* Association of Secretory Component with IgA.** A Madin–Darby canine kidney cell line (MDCK) transfected with the high molecular weight membrane form of rabbit SC, also called the polymeric immunoglobulin receptor, was kindly provided by K. E. Mostov (Whitehead Institute, Cambridge, MA) (25). Approximately 1×10^6 of these cells, designated pWe, were cocultured with 2×10^6 cells of each of the IgA-secreting transfectomas in 25 ml of medium in a 75-cm^2 tissue culture flask (Falcon) for 5 days. The TCFs were concentrated 25 times and immunoprecipitated with anti-rabbit α-chain antiserum and *S. aureus*. The immunoprecipitates were analyzed by immunoblots developed with ^{125}I-labeled anti-rabbit SC. The pWe cell culture fluid was immunoprecipitated with anti-rabbit SC and *S. aureus*, and it served as a positive control.

RESULTS

Expression of 12 C_α Genes and Biochemical Analysis of the IgAs. Each of 13 germ-line C_α genes, $C_\alpha 1$–$C_\alpha 13$, was subcloned into the expression vector and transfected into the SVCK cell line. The TCFs from stable transfectomas were analyzed by ELISA for DNS-binding rabbit–mouse chimeric IgA antibodies. Twelve of the 13 transfectomas, $C_\alpha 1$, $C_\alpha 2$, $C_\alpha 3$, $C_\alpha 4$, $C_\alpha 5$, $C_\alpha 6$, $C_\alpha 7$, $C_\alpha 8$, $C_\alpha 9$, $C_\alpha 10$, $C_\alpha 11$, and $C_\alpha 13$, secreted DNS-binding IgA antibodies. The $C_\alpha 12$ construct was transfected on three different occasions, but no DNS-binding antibody was detected in >250 mycophenolic acid-resistant clones. Thus, all of the cloned C_α genes, except $C_\alpha 12$, were expressible.

Samples from each of the 12 IgA-producing transfectomas were analyzed by $NaDodSO_4$/PAGE and protein immunoblots. The TCFs immunoprecipitated with anti-rabbit IgA antiserum revealed α chains ranging in size from ≈60 kDa for $C_\alpha 1$ to 72 kDa for $C_\alpha 5$ (Fig. 1). Similar analysis (data not shown) of transfectoma cell lysates immunoprecipitated with anti-rabbit IgA antiserum showed that the α heavy chains in the lysates were 2–12 kDa smaller than those in the corresponding TCFs (Table 2). The increased size of the α chains in the TCFs, relative to those in the lysates, indicates that posttranslational modification of the α chains has occurred before their secretion.

Serologic Analysis of Transfectoma IgA with Anti-IgA-f and Anti-IgA-g Antisera. The anti-DNS antibody from each of the 12 IgA-producing transfectomas was reacted with anti-IgA-f or anti-IgA-g alloantisera. Since $C_\alpha 5$ and $C_\alpha 6$ were cloned from rabbit DNA of the *C* heavy-chain haplotype (IgA allotypes f72 and g74), the $C_\alpha 5$ and $C_\alpha 6$ TCFs were tested for reactivity with anti-f72 and anti-g74 antisera. All of the other C_α genes were cloned from rabbit DNA of the *G* heavy-chain haplotype (IgA allotypes f71 and g75); these C_α TCFs were tested for reactivity with anti-f71 and anti-g75 antisera. The results demonstrated that IgA molecules from the $C_\alpha 13$

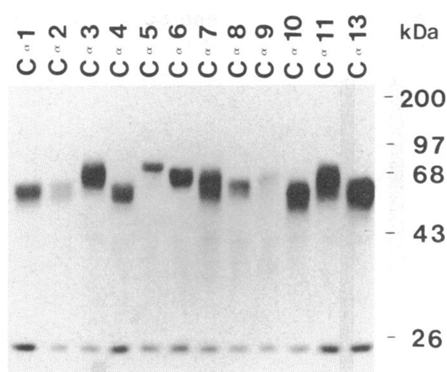

FIG. 1. Immunoblot of transfectoma IgA. Anti-rabbit IgA immunoprecipitates of the TCFs were reduced, electrophoresed on NaDodSO$_4$/10% polyacrylamide gels, and electroblotted onto nitrocellulose membranes. The blot was developed with a mixture of ^{125}I-labeled anti-rabbit α chain and anti-mouse immunoglobulin antibodies and autoradiographed. The lanes are marked to identify the corresponding IgA transfectomas. Molecular size standards (Bethesda Research Laboratories) are myosin heavy chain, 200 kDa; phosphorylase b, 97 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; α-chymotrypsinogen, 26 kDa.

transfectoma reacted with anti-IgA-g but not with anti-IgA-f antibodies (Fig. 2 *Top* and *Middle*). The IgA molecules from each of the other 11 transfectomas reacted with anti-IgA-f, but not with anti-IgA-g antibodies (Fig. 2). TCFs from the parental transfectant SVCK did not react with any of these alloantisera. Thus, 11 of the C_α genes, designated $C_\alpha1$-*f*, $C_\alpha2$-*f*, $C_\alpha3$-*f*, $C_\alpha4$-*f*, $C_\alpha5$-*f*, $C_\alpha6$-*f*, $C_\alpha7$-*f*, $C_\alpha8$-*f*, $C_\alpha9$-*f*, $C_\alpha10$-*f*, and $C_\alpha11$-*f*, encode C_α-*f* heavy chains; a single C_α gene, $C_\alpha13$-*g*, encodes C_α-*g* heavy chains.

***In Vitro* Association of Secretory Component with the Chimeric IgAs.** In previous studies, it was shown that SC is covalently bound to sIgA-f molecules and noncovalently bound to sIgA-g molecules (5). In the current studies, the binding of SC to the newly identified IgA isotypes was investigated by coculturing each of the 12 IgA-producing transfectomas with the pWe cell line and analyzing the culture fluid IgA. This *in vitro* system mimics the *in vivo* situation of polymeric immunoglobulin receptor-mediated transcytosis insofar as the dimeric IgA reacts with the membrane-bound 120-kDa polymeric immunoglobulin receptor and is endocytosed, ultimately resulting in the secretion of dimeric IgA associated with SC (sIgA, ≈400 kDa) (26). Cell culture fluids from the cocultures were immunoprecipitated with anti-rabbit IgA antiserum, and the immunoprecipitates were analyzed by NaDodSO$_4$/PAGE and immunoblots. The NaDodSO$_4$/PAGE was run under nonreducing conditions and the blots were reacted with anti-rabbit SC antibody. For

Table 2. Comparison of the sizes of the α chains immunoprecipitated from the cell lysates and TCFs

Transfectoma	Lysate, kDa	TCF, kDa	Size difference, kDa
$C_\alpha1$	58	60	2
$C_\alpha2$	59	61	2
$C_\alpha3$	56	68	12
$C_\alpha4$	56	60	4
$C_\alpha5$	62	72	10
$C_\alpha6$	62	68	6
$C_\alpha7$	59	62	3
$C_\alpha8$	58	62	4
$C_\alpha9$	60	66	6
$C_\alpha10$	56	60	4
$C_\alpha11$	58	65	7
$C_\alpha13$	58	60	2

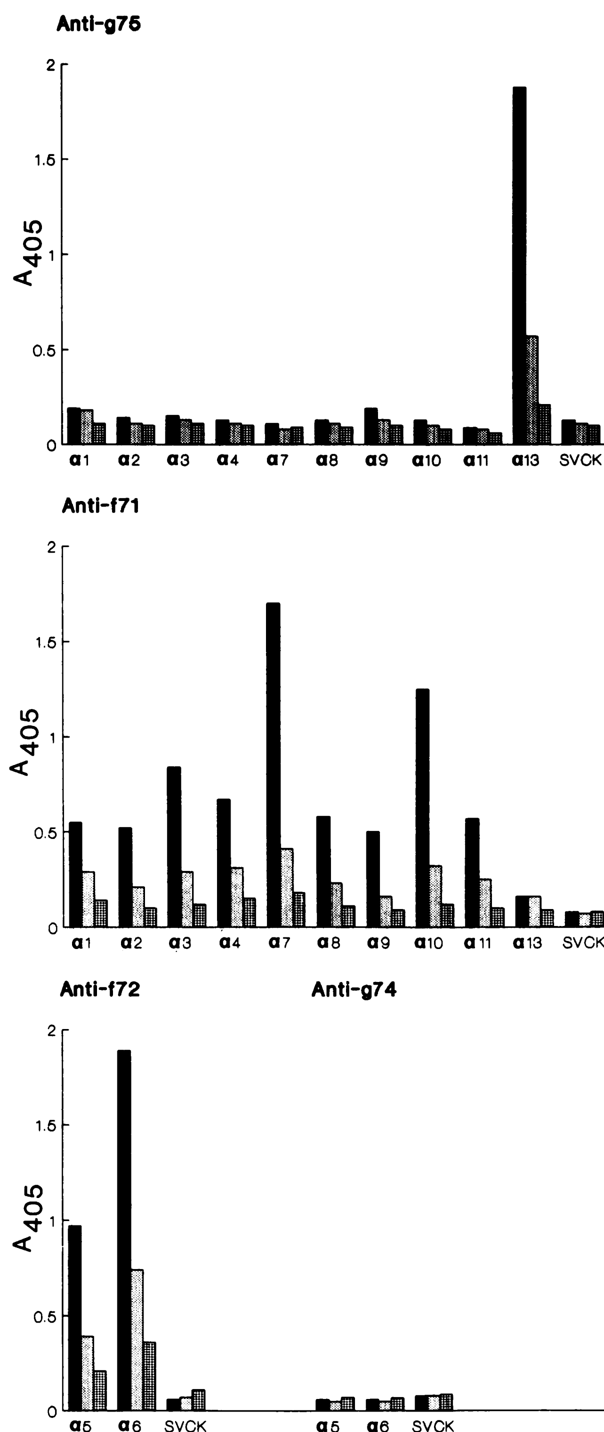

FIG. 2. Reactivity of TCF IgA with anti-IgA-f and anti-IgA-g antisera in an ELISA. The $C_\alpha1$, $C_\alpha2$, $C_\alpha3$, $C_\alpha4$, $C_\alpha7$, $C_\alpha8$, $C_\alpha9$, $C_\alpha10$, $C_\alpha11$, and $C_\alpha13$ TCF IgA samples were reacted with anti-g75 (*Top*) and anti-f71 (*Middle*) antibodies. The $C_\alpha5$ and $C_\alpha6$ transfectoma IgA samples were reacted with anti-f72 and anti-g74 antibodies (*Bottom*). The dilutions of the TCFs shown are as follows: solid bar, undiluted; shaded bar, diluted 1:4; stippled bar, diluted 1:16.

those isotypes that bind SC covalently, we expected the anti-SC to react with molecules of ≈400 kDa on the immunoblot. The blot (Fig. 3 *Upper*) shows that anti-SC reacted with a molecule of ≈400 kDa, for each of the IgA transfectomas except $C_\alpha13$-g. This indicates that SC was covalently bound to the dimeric IgA secreted by all of those transfectomas. To further demonstrate the nature of this association, we subjected the coculture IgA immunoprecipitates to re-

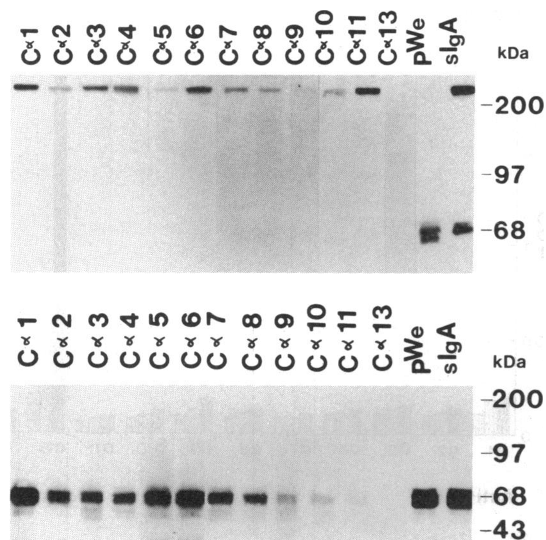

FIG. 3. Immunoblots of *in vitro* associated IgA and SC from cocultures of IgA transfectomas and pWe cells. Anti-IgA immunoprecipitates of the coculture fluids were electrophoresed on $NaDodSO_4$/7.5% polyacrylamide gels. The gels were electroblotted onto nitrocellulose membranes and the blots were developed with ^{125}I-labeled anti-rabbit SC antibody. Immunoprecipitates were analyzed in the absence (*Upper*) or presence (*Lower*) of 2-mercaptoethanol. Each lane is marked to identify the corresponding IgA transfectoma or control. The lane marked pWe is an anti-SC immunoprecipitate of the cell culture fluid from pWe cultures; the lane marked sIgA contains purified rabbit colostral sIgA. Molecular size standards are the same as in Fig. 1.

duction prior to their analysis by $NaDodSO_4$/PAGE and immunoblot. For each of these IgA isotypes, the band of reactivity with anti-SC shifted from a molecule of 400 kDa to a molecule of 68 kDa (Fig. 3 *Lower*), thereby demonstrating that SC had been associated with IgA by disulfide bonds.

Immunoblots of IgA from the cocultures of C_α13-g transfectoma and pWe cells showed no reaction with anti-SC antibodies (Fig. 3). The ability of the C_α13-g IgA molecules to associate with SC was tested in an ELISA. The fluid from the coculture of the C_α13-g transfectoma with the pWe cells was incubated on DNS-coated ELISA plates and the captured IgA was reacted with biotinylated anti-rabbit SC antibodies. The results demonstrate that SC could be detected by the ELISA (Fig. 4), thereby confirming the ability of C_α13-g IgA to associate with SC.

DISCUSSION

The complexity of rabbit IgA has been recognized for several years, but in-depth structural and functional analyses of this system have been hampered by the lack of rabbit B-cell neoplasias and stable hybridomas secreting rabbit immunoglobulin. The identification and cloning of 13 C_α genes combined with the development of transfectomas have now provided monoclonal IgA antibodies for characterization of rabbit IgA. Twelve of the 13 nonallelic germ-line C_α genes were found to be expressible, implying that rabbit IgA may be composed of as many as 12 IgA isotypes. Although the transfected $C_\alpha 13$ gene was a hybrid of the $C_\alpha 8$ and $C_\alpha 13$ genes, we believe that the complete $C_\alpha 13$ gene is expressible because the nucleotide sequence of $C_\alpha 13$ (R. C. Burnett and K.L.K., unpublished results) is identical to that of a previously described cDNA clone (27). The lack of expression of the one gene, $C_\alpha 12$, suggests that it is a pseudogene; however, it could be that $C_\alpha 12$ is functional but was rendered inexpressible during subcloning. Even though we have

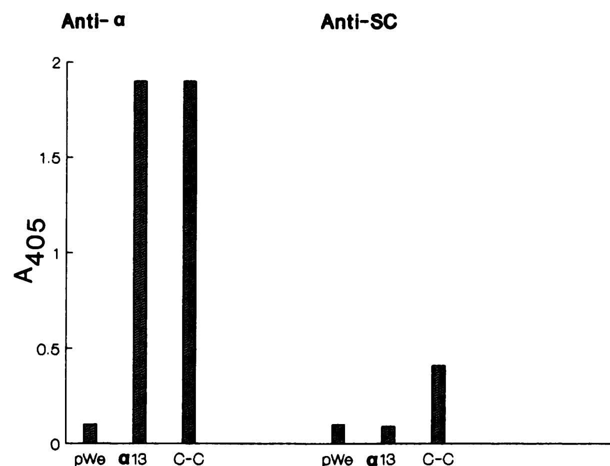

FIG. 4. Reactivity of C_α13-g IgA from cocultures (C-C) of pWe and C_α13-g cells with anti-α-chain or anti-SC antibodies in an ELISA. The IgA from the coculture fluid was captured on ELISA plates coated with DNS-gelatin. Cell culture fluids from pWe cells and from C_α13-g transfectomas were used as controls.

shown that 12 of the germ-line C_α genes are expressible in the transfectomas, we do not know if all 12 are expressed *in vivo*. From serologic studies with colostral IgA (9), we expect that at least four C_α genes are expressed. The *in vivo* expression of the 12 C_α genes needs to be investigated but must await development of either isotype-specific antibody probes or gene-specific RNA/DNA probes.

Serologic analysis of the IgA transfectomas showed that 11 of the 12 expressed C_α genes encode molecules that react with the previously defined anti-IgA-f antibodies. This indicates that the *in vivo* expressed IgA-f molecules, as defined by the original anti-IgA-f antibodies, could be composed of as many as 11 different IgA heavy-chain isotypes. In contrast, the IgA-g molecules appear to be a single isotype with the C_α region of the heavy chain encoded by $C_\alpha 13$. That the 11 IgA-f α-chain isotypes are different from one another is known from restriction maps and from nucleotide sequence analyses of the cloned C_α genes (R. C. Burnett and K.L.K., unpublished results). In the rabbit, 30–60% of total sIgA molecules are IgA-g (4); therefore, $C_\alpha 13$-*g* must be expressed at high levels. The IgA-f molecules represent 40–70% of the sIgA molecules and could be encoded by up to 11 C_α genes. Thus, expression of the C_α genes must be differentially regulated.

The α chains of the secreted transfectoma IgAs ranged in size from 60 to 72 kDa. The amino acid sequence deduced from the nucleotide sequences of the C_α genes shows that the 12 C_α polypeptide chains differ by, at most, 2 kDa (R. C. Burnett and K.L.K., unpublished results). Thus, the large differences in size among the α chains of the secreted IgA molecules are not due to protein but are most likely due to carbohydrate. It would be important to confirm this observation by examining the α chains after their treatment with glycosidases or after culture of the transfectomas in the presence of tunicamycin to inhibit N-linked glycosylation. The different extent of glycosylation of the various α chains could be significant in that the carbohydrate moieties may influence the biologic effector function(s) of the different IgA isotypes.

In previous studies in which immunochemically separated IgA-f and IgA-g molecules from colostrum were used, we demonstrated that IgA-f molecules bind SC covalently and that IgA-g molecules bind SC noncovalently (5). In those studies, however, the IgA-f molecules were most likely composed of a mixture of several IgA-f isotypes. In the present studies, each of the 12 IgA isotypes was tested for its ability to bind SC. Since the SP2/0 myeloma cells used for the

transfections synthesize J chain (28), it was thought that the transfectomas would secrete dimeric IgA and, as such, would be able to bind SC. In fact, immunoblots of TCF from each transfectoma revealed that dimer IgA molecules had been secreted (unpublished data). Each of the 11 IgA-f isotypes was shown to bind SC covalently when IgA immunoprecipitates were made from coculture fluids of the transfectoma and pWe cells and analyzed by $NaDodSO_4$/PAGE and immunoblots. On the other hand, SC binding to the C_α13-g IgA molecules could not be detected by this type of analysis. This was the expected result based on the assumption that C_α13-g IgA molecules bind SC by noncovalent bonds only. Because the K_a for the noncovalent association of rabbit IgA and SC is $\approx 10^8 M^{-1}$ (29) and the concentrations of the reactants in the coculture fluids are in the ng/ml range [i.e., $<10^{-10}$ M (unpublished results)], the reactants in the fluid phase would remain predominantly in the dissociated state. Since the binding of SC to C_α13-g IgA could be demonstrated by a solid-phase assay, noncovalent binding of these species is implied. This noncovalent association indicates that the IgA-g α chains must be structurally different from the IgA-f α chains, as well as from human and mouse α chains, all of which bind SC covalently (1). The simplest explanation for the structural difference is that IgA-g α chains have an amino acid substitution for a cysteine residue involved in the disulfide bonding of SC to α heavy chains. Nucleotide sequence data of each of the 13 C_α genes has not, however, identified such a residue. The molecular basis for the different mode of SC binding to the IgA-f and IgA-g molecules remains to be elucidated. Previous amino acid and nucleotide sequence studies had shown that IgA-g heavy chains of the g75 allotype have serine substituted for the Cys-299 and Cys-311 residues, which are conserved in mouse and human α chains (27). If the covalent association of SC to IgA is mediated by cysteine at one or both of these positions, we would expect that all of the C_α-f genes encode cysteine at one or both of these positions.

The rabbit is unusual in that it has multiple IgA isotypes and only one IgG isotype (4, 10). The multiple IgAs may have evolved to carry out effector functions that in other species are carried out by multiple IgG isotypes. The IgA isotypes may also have evolved to protect the anatomically and functionally unusual gastrointestinal tract of rabbit. Lagomorphs have an unusually large cecum, harboring a large bacterial load; the bacterially modified contents of the cecum are excreted as soft pellets, which are reingested. This process may subject the entire gastrointestinal tract to an unusually large quantity and/or diverse array of microorganisms. It is known that some human IgA molecules are susceptible to cleavage by bacterial proteases (30, 31), and it is possible that the multiple rabbit IgA isotypes have evolved to ensure that at least some of them would be resistant to cleavage by bacterial proteases produced in the rabbit gut.

Our ability to express individual rabbit IgA C_α genes provides a unique opportunity to study the structure and function of the different IgA isotypes and the control of their gene expression. The results of such studies may provide additional insight into the selective pressures operating in the evolution of this complex immunoglobulin gene family.

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