Light chain-deficient mice produce novel multimeric heavy-chain-only IgA by faulty class switching

Louise S. Matheson*, Michael J. Osborn*, Jennifer A. Smith, Daniel Corcos, Maureen Hamon, Rima Chaouaf, John Coadwell, Geoff Morgan, David Oxley and Marianne Brüggemann

The Babraham Institute, Babraham, Cambridge CB22 3AT, UK

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Abstract

Recently, we identified that diverse heavy chain (H-chain)-only IgG is spontaneously produced in light chain (L-chain)-deficient mice (L−/− with silenced κ and λ loci) despite a block in B cell development. In murine H-chain IgG, the first Cγ exon, Cγ1, is removed after DNA rearrangement and secreted polypeptides are comparable with camelid-type H-chain IgG. Here we show that L−/− mice generate a novel class of H-chain Ig with covalently linked α chains, not identified in any other healthy mammal. Surprisingly, diverse H-chain-only IgA can be released from B cells at levels similar to conventional IgA and is found in serum and sometimes in milk and saliva. Surface IgA without L-chain is expressed in B220+ spleen cells, which exhibited a novel B cell receptor, suggesting that associated conventional differentiation events occur. To facilitate the cellular transport and release of H-chain-only IgA, chaperoning via BiP association seems to be prevented as only α chains lacking CH1 are released from the cell. This appears to be accomplished by imprecise class-switch recombination (CSR) from Sm into the α constant region, which removes all or part of the Ca1 exon at the genomic level.

Introduction

IgA plays a central role in mucosal immunity, which is established after its release from a plasma cell and transport to the mucosal epithelial cell layer. Here polymeric IgA is bound to the polymeric Ig receptor, which, after cleavage, provides the secretory component important for stabilization and conferring resistance to attack by proteases (reviewed in ref. 1). Secretory IgA contains two H2L2 units joined by one J-chain (2) and is generally abundant in secretions such as milk and colostrum. With regard to serum IgA, levels are lower in the mouse with the dimeric form common, whereas in humans, it is more highly expressed but monomeric.

In healthy individuals, single-Ig heavy chains (H-chains) cannot be released from the cell because intracellular transport of Ig is only achieved upon correct folding and assembly in the endoplasmic reticulum (ER). A single H-chain of any isotype is chaperoned by association with the H-chain-binding protein, BiP or grp78 (3), which is then displaced by light chain (L-chain), allowing translocation to the cell surface or secretion. However, the release of single-chain IgA is observed in human α-H chain disease (HCD), an immunoproliferative disorder prevalent in developing countries (4), accompanied by rapid expansion of B cells producing truncated α H-chain (5). In this case, removal of Vα1 and/or Ca1 exons precludes BiP association, facilitating translocation and secretion.

In camelids (camels, dromedaries and llamas), and recently in mice, it has been shown that IgG antibodies without L-chain are expressed when the Cγ1 exon is removed by splicing of the RNA transcript or DNA deletion, respectively (6–8). The loss of this exon fits with its function of providing a disulfide linkage to the L-chain. Parallels have been drawn to the expression of H-chain-only antibodies in cartilaginous fish, which also lack Cγ1 or a Cγ1-type domain (9, 10). These single chains are comprised of a flexible assembly of 3–5 Cμ domains and are part of a large assortment of isotypes of different lengths and function found in lower vertebrates, possibly arising by differential splicing to overcome proteolysis (11).

Here we describe a new type of antibody, H-chain-only IgA, and compare its features and expression in relation to developmental processes in health and disease. Reverse transcription (RT)–PCR and protein analysis revealed extensive diversity in its VμDJμ usage, and DNA analysis...
indicated that genomic alterations linked to class-switch recombination (CSR) are responsible for allowing its expression. Understanding the constraints of H-chain IgA, in terms of its expression and function, may help to explain why particular features of the conventional antibody were favored in evolution to provide immune protection (12, 13).

Materials and methods

Mouse strains

The derivation of Igκ- and Igλ-deficient (L–/–, with Cκ disrupted by neo insertion and a ~120 kb region from Cλ2 to Cλ1 removed by targeted integration and Cre-loxP deletion), C deletion (CΔ, lacking a ~200 kb region from Cκ to 3′ of Cκ removed by targeted integration and Cre-loxP deletion) and μMT mice has been described (14–16). L–/– and μMT animals were cross-bred to homozygosity. Mice ranging from 2 to 14 months old were analyzed. Animals were housed at the Babraham Institute in an open or closed (barrier) facility and procedures were carried out under project license PPL 80/1872 under regulations of the UK Home Office.

ELISA of body fluids

Serum antibodies were analyzed as described (17) on Falcon plates coated with 15 μg ml−1 anti-mouse IgA (Sigma M 8769, Sigma-Aldrich, Dorset, UK). Biotinylated detection antibody, anti-mouse IgA (Sigma B 2766), was used at a 1:300 dilution, followed by incubation with 1:300 streptavidin-biotinylated HRP (Amersham RPN 1051V, GE Healthcare, Buckinghamshire, UK). Antibodies in milk, saliva, urine and feces were analyzed in the same way. For this, a known mass (weight per volume) of fecal or milk (taken from the stomachs of pups) sample was dissolved in an equivalent volume (50–100 μl) of PBS. Saliva from swabs was also taken into PBS (50 μl).

Western analysis and mass spectrometry

Serum Ig was captured on anti-mouse Ig (μ, γ and κ H-chain specific; SouthernBiotech 1010-01, Cambridge BiScience, Cambridge, UK) or, for mass spectrometry (MS), anti-mouse IgA (Sigma M 8769)-coupled Sepharose, separated on Ready Gels (161-1155, Bio-Rad, Hemel Hempstead, UK) under reducing or non-reducing conditions and transferred to nitrocellulose membranes as described (18). Filters were incubated with biotinylated [biotin (BIO)] anti-mouse antibodies specific for IgA (Sigma B 2766) or Igκ (04-6640, Zymed, SelectScience, Bath, UK) and λ (02172D, BD PharMingen, Oxford, UK) L-chain. This was followed by incubation with streptavidin-biotinylated HRP (RPN1051V, Amersham) and visualization of bands using SuperSignal West Pico Chemiluminescent reagent, reduced, alkylated and digested overnight with 10 ng ml−1 Sequencing Grade Modified Trypsin (9PIV5113, Promega, Southampton, UK) in 25 mM NH4HCO3 at 30°C. The resulting peptide mixtures were separated by reversed-phase nanoscale liquid chromatography (nanoLC) with online MS analysis as previously described (8). Proteins/peptides were identified by database searching of the mass spectral data using Mascot software (Matrix Science, London, UK). For the identification of J-chain, the total immunoprecipitated protein was reduced, alkylated, digested with trypsin and analyzed by nanoLC-MS as described above. The presence (or otherwise) of J-chain was confirmed by manual inspection of extracted ion chromatograms of the ions of m/z 578.8 (2+), 461.2 (3+), 792.3 (2+) and 576.3 (3+) corresponding to the J-chain peptide CYTTMoxVPLR, NFVYHLDSDKV, MoxVQAATLPSCYPD and IIPSTEDPNEDIVER, respectively.

Flow cytometric analysis and cell sorting

Multi-color analyses and sorting were carried out on a BD FACSria. For analysis of IgA-positive cells, spleen cell suspensions were prepared and cells were stained with anti-mouse IgA-BIO (Sigma), CD45R (B220)-allophycocyanin, CD90-fluorescein isothiocyanate (FITC) (553092 and 553013, BD PharMingen) and F4/80-FITC (11-4801-82, eBioscience, San Diego, CA, USA). This was followed by incubation with PE–Cy5.5-conjugated streptavidin (35-4317, eBioscience). FlowJo software was used for the analysis. For sorting of plasma cells for genomic DNA analysis, spleen cells were stained with FITC-conjugated anti-CD45R and BIO-conjugated anti-CD138 (syndecan-1) (553270 and 553713, BD PharMingen) followed by incubation with PE–Cy5.5-conjugated streptavidin (eBioscience).

RT-PCR analysis

RNA was isolated from tissue or cells using Trizol (Gibco BRL, 15596-026, Invitrogen, Paisley, UK) and reverse transcribed at 42°C with Omniscript (205111, Qiagen, Crawley, UK) or Bioscript (BIO-27036, Bioline, London, UK) reverse transcriptase. PCR reactions with Jκ and Ca3 primers were set up using KOD Hot Start DNA Polymerase (71086-4, Novagen, UK). For the identification of J-chain, the total immunoprecipitated protein was reduced, alkylated, digested with trypsin and analyzed by nanoLC-MS as described above. The presence (or otherwise) of J-chain was confirmed by manual inspection of extracted ion chromatograms of the ions of m/z 578.8 (2+), 461.2 (3+), 792.3 (2+) and 576.3 (3+) corresponding to the J-chain peptide CYTTMoxVPLR, NFVYHLDSDKV, MoxVQAATLPSCYPD and IIPSTEDPNEDIVER, respectively.

Genomic DNA analysis

Genomic DNA was prepared and analyzed by long-range PCR as described previously (8). A first-round PCR of 20 cycles from Jκ4L to Ca2L1 was followed by a nested second-round PCR of 25 cycles from 3′Eμ to Ca2L2
(primers listed in Supplementary Table S1, available at International Immunology Online). Any bands obtained were cloned as described above or picked from the gel and re-amplified and sequenced.

Results

Expression of high levels of IgA in L−/− mice

The recent discovery that H-chain-only IgG, similar to cam-elid single-chain IgG, is produced in L−/− mice (8) prompted further analyses to identify, or rule out, the presence of other isotypes. ELISA discovered IgA titers in serum from L−/− mice, which in some cases were very similar to the level of conventional IgA found in normal mice (Fig. 1A); in comparison with the low levels of IgG expression seen in L−/− mice, it appears that H-chain IgA can be produced much more readily. This antibody class is not known to be secreted without L-chain in healthy animals; however, the well-being of the L−/− mice appears to be unaffected.

Size and configuration of H-chain-only IgA

To determine the molecular weight of the α H-chains, western blot analysis was performed on Ig separated under reducing and non-reducing conditions (Fig. 1B and C, respectively). This shows α H-chains of ~46 kDa, which is ~15 kDa or one domain shorter than conventional α chains (Fig. 1B). H-chain IgA appears to share a common feature with H-chain IgG, in that the size of the H-chain is approximately one domain shorter than normal in both cases. The analysis of purified IgA polypeptides from gel slices by MS was consistent with the lack of a single domain, as it revealed an extensive number of Cα sequences from Cα2 and Cα3, but not Cα1 [Supplementary Table S2(A), available at International Immunology Online]. Separation under non-reducing conditions (Fig. 1C) identified covalent linkage of two α H-chains with a molecular weight of ~92 kDa and a low level of multimers, four covalently linked α H-chains, similar to multi-chain normal IgA (1). We were not able to identify covalent J-chain association, either in ELISA or western blots, due to a lack of specific reagents. Its presence would add ~15 kDa to the molecular weight of the tetramer; however, this small difference cannot be resolved in the high-molecular weight region of the gel.

To clarify whether J-chain is attached to H-chain-only IgA, serum IgA from L−/− mice was captured with anti-IgA coupled to Sepharose and the mixture was directly analyzed by MS. This revealed truncated α H-chain- as well as J-chain-specific peptides [Supplementary Table S2(B), available at International Immunology Online]. However, additional peptides from other serum proteins were also identified (not shown), raising the possibility that J-chain could be released unassociated.

Lymphoid tissues express truncated α H-chain

The production of α H-chain transcripts in different tissues was compared by semi-quantitative RT-PCR (Fig. 2A). In normal mice, transcripts from JH to Cα3 are ~850 bp, whereas in L−/− mice, the predominant band was ~550 bp. The use of different JH oligos, for JH1, 2, 3 and 4, revealed the smaller product in all amplifications using RNA from bone marrow and spleen. Sometimes, the ~550-bp band was also obtained from lymph node and ileum preparations, for example in JH2 to Cα3 reactions. Occasionally, an ~850-bp product was seen, presumably corresponding to a normal size transcript; however, these signals were always weaker, indicating that the shorter product represents the major product in these tissues. Sequencing revealed that the shorter product encompasses a region from JH4 to Cα3 without Cα1.

Diverse VH, D and JH usage in H-chain IgA

The analysis was extended to gain information about the VH gene repertoire of H-chain-only antibody transcripts. Amplification with different VH family oligos (J558, VGAM and V7183) identified strong bands of ~880 bp, representing α H-chains encompassing VHr-D-JHr-Cα2-Cα3 but not Cα1.
similar products were also seen upon amplification with the more degenerate \( V_H \) gene primer, Vgen (data not shown). These products were cloned and sequenced, allowing identification of several different \( V_H \), D and \( J_H \) segments from each mouse and also showing that \( J_H \) is correctly spliced to \( C_H2 \) and in one case to \( C_H3 \) (Supplementary Table S3, available at International Immunology Online). The full-length product of \( \sim 1150 \) bp was the main band found in normal mice. However, a truncated product, represented by a smaller band of weaker intensity, was also present and may indicate a spontaneous mechanism to produce truncated \( \alpha \)-H-chains.

Analysis of the \( V_H \) domain showed diverse D and \( J_H \) rearrangement with the addition of non-encoded residues at the junctions and extensive alterations by hypermutation (Supplementary Tables S3 and S4, available at International Immunology Online). Interestingly, nearly half (10/22) of the \( V_H \) sequences carry a high level of replacement residues, suggesting antigen-dependent selection, and the high ratio of transition to transversion mutations is a well-established feature of the somatic hypermutation mechanism (20, 21).

Translation of the RT–PCR sequences illustrates the possible extent of amino acid changes, many with multiple alterations (10/20 \( V_H \)-D-\( J_H \)s with 4 or more mutations and up to 17 alterations in one sequence). The \( V_H \) alignment program ClustalX (http://www.ebi.ac.uk/Tools/clustalw2/index.html) (22) was used to identify similarities in the configuration of \( V_H \) sequences from the L\(^{-/-}\) mice with a rearranged llama \( V_H \) sequence of known crystallographic structure (23). This revealed that only the extensively mutated \( V_H \) gene \( V1S128*01 \) contained one of the \( V_H \) hallmark amino acids, arginine, at position 48 (Supplementary Table S4, available at International Immunology Online). The \( V_H \) structures were also modeled using SWISS-MODEL (http://swissmodel.expasy.org/SWISS-MODEL.html). This suggested that in most cases, the interface usually interacting with the L-chain does not contain mutations that would prevent this association (data not shown). The diversity of \( V_H \) genes used in the H-chain-only antibodies was confirmed by the results of MS: \( V_H \) sequences from four different families, \( VH7183, J558, VGAM3 \) and 3609, were identified by their framework and complementarity determining regions (CDRs) [see Supplementary Table S2(A), available at International Immunology Online].

To further investigate whether antibody specificities could potentially be selected, we stained the surface of spleen cells with anti-IgA. As can be seen in Fig. 3, after T cell and macrophage exclusion, a small but distinct population of IgA\(^+\)B220\(^+\) cells (1.4%) can be identified. This population could not be readily distinguished in all animals analyzed; however, when mice displaying higher serum IgA levels (LN, peritoneum (PE), thymus (TH), ileum (IL) and kidney (Ki)) from L\(^{-/-}\) mice and spleen from normal mice (NM SP). \( \alpha \)-H-chain bands of reduced size, \( \sim 550 \) bp, are present in lymphoid tissues from L\(^{-/-}\) mice, sometimes accompanied by the full-size product of \( \sim 850 \) bp, typical for normal mice. \( \beta \)-Actin served as a control to ascertain the use of equal amounts cDNA template. (B) \( V_H \) (J558, VGAM and V7183 oligos) to \( C_H3 \) amplification of NM and L\(^{-/-}\) spleen c-DNA shows extensive \( V_H \) gene usage in shorter products (\( \sim 880 \) bp, bottom arrow) compared with normal products (\( \sim 1150 \) bp, top arrow).

![Fig. 2](image-url) Identification of reduced size \( \alpha \) transcripts in lymphoid tissues. (A) RT-PCR amplification from \( J_H1, J_H2, J_H3 \) or \( J_H4 \) to \( C_H3 \) using RNA prepared from total bone marrow (BM), spleen (SP), lymph nodes (LN), peritoneum (PE), thymus (TH), ileum (IL) and kidney (Ki) from L\(^{-/-}\) mice and spleen from normal mice (NM SP). \( \alpha \)-H-chain bands of reduced size, \( \sim 550 \) bp, are present in lymphoid tissues from L\(^{-/-}\) mice, sometimes accompanied by the full-size product of \( \sim 850 \) bp, typical for normal mice. \( \beta \)-Actin served as a control to ascertain the use of equal amounts cDNA template. (B) \( V_H \) (J558, VGAM and V7183 oligos) to \( C_H3 \) amplification of NM and L\(^{-/-}\) spleen c-DNA shows extensive \( V_H \) gene usage in shorter products (\( \sim 880 \) bp, bottom arrow) compared with normal products (\( \sim 1150 \) bp, top arrow).
titers were selected, the data were found to be reproducible. As no conventional L-chain is produced in these mice (8, 14), this is the first example of spontaneous expression of a new type of B cell receptor (BCR) without L-chain on mature B cells.

H-chain IgA secretion and excretion

The varied H-chain-only IgA titer in L⁻/⁻ mice prompted a detailed analysis of age-related and environmental constraints which could drive expression. The questions we addressed were as follows: Do older animals produce higher Ig levels and does an open or closed, pathogen-free, animal facility bias the expression? A comparison of IgA levels at 100-fold serum dilution is presented in Fig. 4, which shows the predicted range for conventional IgA from normal mice. Some of the older L⁻/⁻ animals housed in the closed facility, 13, 11, 9 and 8 months of age, gave the highest titers very similar to normal mice. Some 5-month-old mice had a medium titer and some younger mice had a low titer, but there were also many exceptions to this pattern. Particularly notable was that several older mice had quite a low titer. However, there does appear to be a propensity favoring higher expression of H-chain IgA in older mice. This trend also occurs in animals kept in open or easily accessible facilities, as only older mice have the highest titer. Overall, the antibody serum levels in open and closed facilities appear to be similar and perhaps other events, for example small injuries or airborne contamination, which may accumulate with time, provide the essential immune stimulation to obtain high antibody titers.

Fig. 3. Surface IgA⁺ lymphocytes in L⁻/⁻ spleen. Flow cytometry analysis of spleen cells from L⁻/⁻ mice compared with normal (NM) mice and constant region deletion (CA) animals. Numbers show the percentage of cells in the respective gate. Gating spleen lymphocytes (left) and exclusion of their T cells and macrophages (middle) identified for L⁻/⁻ mice 1.4% of IgA⁺ B220⁺ lymphocytes (right). The analysis is a representative presentation of one of the L⁻/⁻ animals with high IgA titer as shown in Fig. 1.
In μMT C57Bl/6 animals, IgA is expressed at various levels and seemingly independent of IgM and IgD (24). To test whether H-chain-only IgA can be expressed independently, we crossed L−/− mice with μMT animals to homozygosity. As shown in Fig. 4 (left), no IgA could be identified in the serum of μMT L−/− animals. The lack of IgA and the previous finding that no H-chain-only IgG is produced when Cμ or Cα retains only the last C gene and juxtaposes the rearranged V(D)JCα <10 kb upstream of Cα (26). Figure 6(A) illustrates the gene layout after switching, showing the position of the oligos for the initial PCR amplification from Jα4L to Cα2L1 followed by a further nested PCR amplification with oligos from 3′Eμ to Cα2L2. The nested amplification bands and the sequence information for the indicated bands obtained after cloning are illustrated in Fig. 6B and C. In L−/− mice, small distinct fragments occur and their sequences showed recombination events between Sμ and Cα, which in most cases removed all or part of Cα1. In some sequences, multiple recombination events have taken place, sometimes switching to Sγ before the joining of Sα and Cα.

Removal of Cα1 by imprecise CSR

A lack of the Cα1 exon, identified in protein and transcriptional analyses of H-chain-only IgA, could be the result of somatic mutation resulting in mis-splicing of RNA transcripts or the generation of large deletions potentially linked to CSR during B cell maturation. We have analyzed genomic DNA from the IgH constant region to identify the occurrence of sizeable deletions by employing a long-range PCR approach using sorted syndecan-positive plasma cells as described recently (8). CSR from Cμ [via Cγ] to Cα retains only the last C gene and juxtaposes the rearranged V(D)JCα <10 kb upstream of Cα (26). Figure 6(A) illustrates the gene layout after switching, showing the position of the oligos for the initial PCR amplification from Jα4L to Cα2L1 followed by a further nested PCR amplification with oligos from 3′Eμ to Cα2L2. The nested amplification bands and the sequence information for the indicated bands obtained after cloning are illustrated in Fig. 6B and C. In L−/− mice, small distinct fragments occur and their sequences showed recombination events between Sμ and Cα, which in most cases removed all or part of Cα1. In some sequences, multiple recombination events have taken place, sometimes switching to Sγ before the joining of Sα and Cα.

To determine if the CSR junctions found in L−/− mice were unusual, we compared them with sequences from normal mice (Fig. 6D and Supplementary Table S5, available at International Immunology Online). No significant difference in the length of donor/acceptor homology at the junctions was found. Also, the mutation frequency in the vicinity of the junctions (±50 bp) was similar. However, intra-switch region recombination was very frequent in the L−/− sequences, in particular within the γ and/or α switch region. The loss of Cα1 was not found in the smaller PCR products from normal mice except in one case. The occurrence of this sequence suggests that the mechanisms leading to exon deletion occur naturally but are selected for in L−/− animals. Thus, it is likely that DNA lesions during CSR (27) facilitate H-chain-only IgA expression.

Discussion

Further study of L-chain-deficient mice has revealed a new type of antibody, H-chain-only IgA, which is released from the cell and surface expressed. There are no examples of the occurrence of this isotype in Tylopoulos or camelids, which produce H-chain-only IgG, or in elasmobranchs (sharks, skates and rays), where H-chain-only antibodies can comprise a variable number of Cμ domains (11, 28). A common feature of murine H-chain-only IgA, as well as other naturally occurring H-chain antibodies, is the lack of a typical Cα1 domain. As a result, the shortened nascent-translated H-chain cannot form an association complex with the H-chain-binding protein BiP as interacting Cα1 residues are lacking (3, 29). This secures unhindered transport through the ER leading to surface deposition and H-chain-only antibody secretion (8, 30). Unexpectedly, H-chain IgA is remarkably stable, degradation seems to be prevented and protein levels are sizeable, in some cases almost reaching conventional IgA levels in the mouse. Flow cytometry and RT-PCR identified spleen lymphocytes as a major
source of α H-chain transcripts lacking Cα1, which is in agreement with the recent findings of short γ transcripts in syndecan+ plasma cells.

The generation of H-chain-only antibodies from truncated transcripts in these mice was initially puzzling. One explanation in the absence of IgM-expressing B cells was through a process of error-prone early class switching (8). Several recent studies have detected CSR in pre-B and immature B cells (31–33). This developmental route would be open to cells in the L−/− mice, which may occasionally result in a large deletion into the coding region of the H-chain C region, and would allow cell surface expression and possible progression to the plasma cell stage. To look for evidence of CSR generated transcripts lacking Cα1, we performed long-range PCR using DNA from sorted spleen cells. Prominent deletions were identified, which appear to be caused by class switching, sometimes containing Sy from an intermediary switching event. An interesting feature of the sequenced switch junctions is the frequent occurrence of intra-switch region recombination. Internal deletions are commonly found in Sp but are rare in other switch regions (34 and references therein). We found an accumulation of deletions in Sα and Sy joined with Sα, accounting for recombination events in over half of the L−/− sequences cloned.

The occurrence of CSR junctions well outside the Sα region in Cα1 or within the intron between Cα1 and Cα2 would be an exceptional event selected for by the absence of the L-chain. Our analysis of genomic DNA from normal mice also revealed the presence of a product lacking Cα1, probably identified because smaller PCR products are more readily amplified. Nevertheless, it does indicate that the mechanism leading to H-chain-only antibody production exists in the normal situation (when L-chains are expressed); this is supported by the presence of a weak lower band in some RT–PCR analyses (see Fig. 2B).

It has been reported that IgA may be expressed independently of IgM or IgD in early ontogeny, which could be an evolutionary primitive system that does not rely on class switching from μ to a downstream isotype (24). As our results are consistent with the notion that H-chain-only IgA is produced and secreted by the same B cell subset as conventional IgA, we asked whether H-chain-only IgA can be expressed in the μMT background, which provides a B cell block due to a lack of surface IgM production (16). This does not seem to be the case and no H-chain IgA or any other isotype has been detected in μMT L−/− animals (see Fig. 4 and ref. 8), which implies that IgM expression during ontogeny is probably essential to progress developmental

Fig. 5. IgA expression in bodily fluids. Excreted IgA in milk, saliva, urine and feces from several normal and L−/− mice was titrated by ELISA. The shading of the bar indicates the dilution (10−1, 10−2 and 10−3). (A) Milk was taken from different pups from the same litter, indicated by a bracket, while other pups were from different mothers. (B) The experiments shown are representative for the finding that only one or two animals of a group of at least five tested in parallel excrete H-chain-only IgA. Animals are not matched except where indicated by an asterisk (*) and this animal is also the mother of the three siblings in (A).
events that allow C gene modification followed by H-chain expression.

In camelids, the V_{\text{HH}} genes used in H-chain-only antibodies often contain specific alterations such as hallmark amino acids or an extended CDR3 region, both to compensate for the lack of L-chain and to prevent L-chain association in a system in which both H-chain-only and conventional antibodies are produced (23, 35–37). Alignment of the V_{\text{H}} sequences from the L^{-/-} mice with a rearranged llama V_{\text{HH}} sequence of known structure (23) identified the V_{\text{HH}} hallmark amino acid arginine at position 48 in one sequence (see Supplementary Table S4, available at International Immunology Online).

However, such alterations appear to be rare, which matches the finding with mouse \( \gamma \) H-chain V_{\text{H}}s and reflects the fact that murine V_{\text{H}}s have not been selected in evolution to be optimal for H-chain-only antibody production. It also suggests that there are fewer restrictions in the sequence of the mouse variable region, probably due to the complete absence of the L-chain in these mice. However, since one function of the V_{\text{H}} hallmark amino acids is to increase the hydrophilicity of the interface (23), the absence of such mutations suggests that there may be sticky patches exposed on the surface of the H-chain-only IgA. For this reason, it may be possible that murine H-chain IgA could be structurally different from the configuration of camelid H-chain IgA. Indeed, certain V_{\text{H}} gene sequences may be advantageous to permit different formats and the capacity of V_{\text{H}} domains to dimerize spontaneously is not unusual (38); modeling of the structures of our V_{\text{H}}s indicated that such a conformation would not be prevented by steric hindrance (data not shown). An associated dimeric configuration of V_{\text{H}} domains has been described (38) and an example of such a linkage binds to DNA (39). Interestingly, conventional monoclonal IgA anti-DNA or auto-antibodies can be readily isolated after fusion of Peyer’s patch cells and key amino acids in their CDR regions have been related to this specificity (40). We have observed that L^{-/-} mice lack visible Peyer’s patches, the factory for IgA produced by the mucosal immune system to combat airborne or food-borne pathogens such as viruses or bacteria, although truncated IgA transcripts have been found in the ileum.

The structural differences between H-chain-only and conventional IgA raised the question of whether the truncated polypeptide, without L-chain, would be recognized by the polymeric Ig receptor (or secretory component), allowing its release from mucosal surfaces. This receptor is produced in epithelial cells separate from plasma cells secreting serum IgA. Its specificity for polymeric Ig (41) implied that the level of IgA secretion into external fluids would be lower in L^{-/-} mice as the extent of IgA oligomerization is reduced. This is indeed the case (see Fig. 5) and our results indicate that while sometimes H-chain-only IgA is found in secretions, this is not usually the case, even when the serum level of IgA is high. H-chain-only IgA therefore appears to be, in general, unable to fulfill one of the central roles of this antibody isotype, impairing mucosal immunity and removing a vital source of neonatal immune protection. Although our finding provides unequivocal evidence that J-chain is released, there remains a possibility that it is not associated or poorly attached to the IgA. However, since IgA polymerization is regulated by J-chain association with the COOH-terminal domain of an \( \alpha \) chain and as this domain is preserved in H-chain-only IgA, it is likely that J-chain can retain its association independent of the C_{\text{H}1} domain and the lack of L-chain.

Previously, production of H-chain-only IgA had only been observed in the context of disease. In L^{-/-} mice, spontaneous expression of H-chain IgA clearly differs from human \( \alpha \)HCD because the animals appear healthy with normal life expectancy in a pathogen-free environment. No invasion of plasma cells has been observed, which would cause lymphomas characteristic of HCD (4). Regular features

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Fig. 6. Long-range PCR identified diverse class-switch-mediated deletions removing Cx1. DNA was prepared from sorted synecdeca* spleen cells (8) from two L^{-/-} mice and one normal animal. (A) The layout of the rearranged and switched (sply/\( \gamma \)/\( \alpha \) J_{\text{H}}) to Cx region, 3–5 kb, is shown with external (black) and nested (shaded) primers indicated by arrows. (B) Gel separation of a nested PCR reaction, from 3'\( \mu \) to Cx2L2 internal, which followed the initial PCR amplification from J_{\text{H}}4 to Cx2L1. (C) Cloning and sequencing of the PCR fragments indicated by thin lines identified complete and incomplete deletions of the Cx proximal switch region and the Cx1 exon (lightly shaded line/boxes). Cx2 is retained and the fragments of 3.0, 2.1, 1.2, 1.1, 1.0 and 0.75 kb correspond to the bands in the gel. Black lines/boxes indicate regions present in each fragment. (D) Alignment of L^{-/-} and normal mouse (NM) genomic sequences identified multiple (\( n \)) switch regions; a typical example (*\( n \)) exhibits multiple breakpoints between \( \mu \), \( \gamma \), and \( \alpha \). All sequences that contained multiple breakpoints are listed in Supplementary Table S4 (available at International Immunology Online).
associated with HCD involve deletions and insertions in the rearranged \( V_\text{H} \) genes (42), neither of which has been found in \( V_\text{H}DJ_\text{H} \) sequences from L\(^{-}\) mice although a more detailed examination will be necessary to confirm this. However, changes to permit cellular transport are common in both systems. In L\(^{-}\) mice, the absence of L-chain leads to the selection and subsequent expansion of cells producing mutant \( \alpha \) H-chains, which have lost the use of C\(_{H1}\).

In summary, we have identified that the endogenous H-chain locus of a mouse B cell has the capacity to develop a remarkable assembly of different H-chain-only isotypes, with diverse multimeric H-chain IgA being a novel class of antibody expressed without L-chain as a BCR and in secreted form.

**Supplementary data**

Supplementary tables are available at *International Immunology* Online.

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BCR</td>
<td>B cell receptor</td>
</tr>
<tr>
<td>BIO</td>
<td>biotin</td>
</tr>
<tr>
<td>CDR</td>
<td>complementarity determining region</td>
</tr>
<tr>
<td>CSR</td>
<td>class-switch recombination</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>HCD</td>
<td>Heavy-Chain Disease</td>
</tr>
<tr>
<td>H-chain</td>
<td>heavy chain</td>
</tr>
<tr>
<td>nanoLC</td>
<td>nanoscale liquid chromatography</td>
</tr>
<tr>
<td>L-chain</td>
<td>light chain</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
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<tr>
<td>PE</td>
<td>phycoerythrin</td>
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<td>RT</td>
<td>reverse transcription</td>
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**References**


