The primary structure of the constant region of Basilea-rabbit immunoglobulin λ -chains

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The amino acid sequence of the constant region of the immunoglobulin λ -chain of the rabbit (C λ) has been determined. This chain was obtained from a homogeneous anti-(type II pneumococcal polysaccharide) antibody fraction raised in Basilea rabbit 4548. The C λ -region sequence was established by the analysis of tryptic and some overlapping chymotryptic peptides and partly by homology with known C λ -region sequences of λ -chains from other species. Peptides were isolated by a combination of methods including high-voltage paper electrophoresis, gel filtration and high-pressure liquid chromatography. The peptides were subjected to automated Edman degradation in the presence of Polybrene. The sequence showed no evidence of heterogeneity. Large interspecies similarities, as well as amino acid interchanges, are apparent among various C λ regions; the degrees of homology between rabbit C λ region and the C λ region from human, porcine and murine chains are 72.6, 71 and 72% respectively. The similarity of λ -chains of different species is much greater than that found between rabbit κ - and λ -chains (about 34% homology). Genetic implications of these results are discussed.

Rabbit immunoglobulin L-chains of the κ type are under the genetic control of the b locus, which determines the synthesis of the allotypic specificities b₄, b₄ variant, b₅, b₆, b₉ and b_{9s} (Oudin, 1966; Mage *et al.*, 1973; Kindt, 1975; Sogn & Kindt, 1976; Benammar *et al.*, 1979). L-chains of the λ type are controlled by the c locus, which determines the C₇ and C₂₁ allotypic specificities (for a review, see Mage *et al.*, 1973). In standard domestic rabbits, λ -chains are a minor component of the IgG pool (Appella *et al.*, 1974). By allotypic suppression of κ -chain synthesis (Dray, 1962), L-chains without b-locus allotype have been obtained and analysed (Appella *et al.*, 1968). Fragmentary structural data obtained with these b-negative L-chains identified them as λ -chains homologous to human and murine λ -chains (Appella *et al.*, 1974; Chersi, 1975). More recently, Kelus & Weiss (1977) described a new rabbit strain, Basilea, characterized by the lack of κ -chains bearing b₄, b₅, b₆ or b₉ markers, which were almost entirely substituted by chains of the λ type (Jaton & Kelus, 1977). Hyperimmunization of

Basilea rabbits with streptococcal (Weiss *et al.*, 1977) and pneumococcal vaccines (Garcia & Jaton, 1979) leads to the production of large amounts of specific antibodies containing L-chains of the λ type. Homozygous *bas/bas* rabbits thus offer a useful source of electrophoretically restricted and even monoclonal λ -chain-bearing antibodies (Weiss *et al.*, 1977; Garcia & Jaton, 1979) in amounts suitable for determining V- and C-region amino acid sequences.

Here we report the complete primary structure of the C-region of λ L-chains derived from a homogeneous *bas* antibody specific for type-II pneumococcal polysaccharide. Partial-amino-acid-sequence studies of these λ -chains have been reported (Garcia & Jaton, 1979).

Experimental

Materials

Trypsin, Tos-Phe-CH₂Cl and α -chymotrypsin were purchased from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Dithiothreitol was from Calbiochem, Lucerne, Switzerland. Iodo[1-¹⁴C]-acetamide (sp. radioactivity 53 mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K., and Polybrene (Hexadimethrine bromide) was from Aldrich Co., Milwaukee, WI, U.S.A.

Abbreviations used: IgG, immunoglobulin G; H- and L-chains, heavy and light chains of IgG; V- and C-regions, variable and constant regions of IgG respectively; h.p.l.c., high-pressure liquid chromatography; Tos-Phe-CH₂Cl, 1-chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK').

Methods

Antibody production and purification. The homozygous Basilea rabbit BS 4548 (generously given by Dr. A. S. Kelus, Basel Institute for Immunology) was hyperimmunized with type-II pneumococcal vaccine as previously described (Brandt & Jaton, 1978a). Serial bleeds were taken and pooled according to the isoelectrofocusing pattern (Braun *et al.*, 1979). Antibody was isolated by chromatography on DEAE-cellulose DE-52 equilibrated in 2 mM-sodium phosphate buffer, pH 7.0. The antibody fraction recovered was subjected to agarose-block electrophoresis (Jaton *et al.*, 1979) to remove faster-migrating antibodies. The purified antibody gave a restricted banding pattern typical of a homogeneous immunoglobulin on isoelectric focusing. The absence of precipitation of *bas* IgG antibody with anti-b₄, -b₅, -b₆ and -b₉ antisera and the formation of a precipitation line in double immunodiffusion with anti-C₇ antisera were taken as serological evidence for the presence of λ L-chains in the antibody fraction (Dray *et al.*, 1963; Mage *et al.*, 1968). The antibody was concentrated to 20 mg/ml and stored at -20°C before use.

Separation of H- and L-chains. Antibody BS 4548 was fully reduced and alkylated with iodo-[1-¹⁴C]acetamide by the technique of O'Donnell *et al.* (1970). The separation of H- and L-chains was performed by gel filtration on a Sephadex G-100 column in 5 M-guanidinium chloride 0.1 M-Tris/HCl buffer, pH 8.3. The L-chain fraction recovered, which accounted for 26–28% of the total protein applied, was extensively dialysed against 1 M-acetic acid and freeze-dried.

Proteolytic digestion of λ L-chains. The fully reduced and 1-¹⁴C alkylated chains were redissolved in 0.05 M-NH₄HCO₃ at a concentration of 5 mg/ml and digested for 5 h at 37°C with 2% (w/w) Tos-Phe-CH₂Cl-treated trypsin. Digestion with α -chymotrypsin was done in the same buffer for 30 min at 37°C. Digests were freeze-dried, redissolved in distilled water and freeze-dried again.

Peptide purification. The tryptic digest was suspended in 0.1% sodium phosphate adjusted to pH 3.0. After 10 min stirring at room temperature, the insoluble fraction of the digest was removed by centrifugation; the pellet was redissolved in 0.02 M-NH₃ and passed through a column (2 cm $\times$ 200 cm) of G-50 Sephadex equilibrium in 0.02 M-NH₃. The flow-through major peak fraction was pooled, freeze-dried and further digested with α -chymotrypsin. The resulting peptides were isolated by high-voltage paper electrophoresis at pH 3.5 on Whatman no. 1 paper. Radioactive fragments were localized by autoradiography for 18 h with Kodak Blue Medical X-ray film.

The soluble fraction of the tryptic digest at pH 3.0 was loaded on a C-18 μ Bondapak reverse-phase

column (3.9 mm $\times$ 300 mm) and the peptides were separated at room temperature by h.p.l.c. on a Waters Associates Instrument (Fullmer & Wasserman, 1979). The initial eluent was 0.01 M-ortho-phosphoric acid adjusted to pH 3.0 with NaOH, followed by a linear gradient of 0–70% (v/v) methanol over 70 min. Peptides were detected spectroscopically at 210 nm and radioactive peptides were monitored by counting a portion of each fraction of the eluate for radioactivity. To achieve better resolution of some peptides, tryptic digests were dissolved in 0.01 M-sodium phosphate, pH 7.0, and the h.p.l.c. was developed in this solvent with a 0–70% methanol gradient over 70 min. Contaminated peptides were fractionated to purity by high-voltage paper electrophoresis at pH 3.5 and/or 6.5.

Amino acid analysis. Peptides were hydrolysed for 22 h at 110°C in sealed tubes *in vacuo*, and hydrolysates were run on a Durrum D-500 amino acid analyser (Benson, 1976).

Amino-acid-sequence determination. Peptides were subjected to automated Edman degradation in a model 890 C Beckman sequencer, with 0.25 M-Quadrol buffer (Garcia & Jaton, 1979) in the presence of 3 mg of Polybrene (Tarr *et al.*, 1978). Identification of each residue was performed as described previously (Brandt & Jaton, 1978b). Most peptides were sequenced twice. The average repetitive yield of Edman degradation ranged from 93 to 95%.

Digestions with carboxypeptidases A and B

Digestions were performed in 0.05 M-NH₄HCO₃ buffer containing about 10–20 nmol of peptide for 30 min at 37°C. Released amino acids were quantified on the amino acid analyser as previously described (Garcia & Jaton, 1979).

Results

Isolation and characterization of peptides from a tryptic digest of [1-¹⁴C]carboxamidomethylated λ -chain 4548

The freeze-dried tryptic digest (about 0.5 μ mol) of λ L-chain was dissolved in 2 ml of 0.01 M-ortho-phosphoric acid, adjusted to pH 3.0 with NaOH. The soluble fraction at pH 3.0, which accounted for about 75% of the total digest, was analysed by h.p.l.c. Six identical runs were performed with about 2 mg of digest. A typical peptide separation is illustrated in Fig. 1. In all, 15 fractions, designated T₁–T₁₅, were recovered, and the fractions from the consecutive runs were pooled and dried under N₂. After desalting on a small Bio-Gel P2 column equilibrated in 1 M-acetic acid, each fraction was subjected to amino acid analysis and partial N-terminal-sequence analysis. Most fractions contained unique peptides that were directly amenable to amino acid

Fig. 1. Elution profile of a tryptic digest of completely reduced and $[1-^{14}\text{C}]$ -carboxamidomethylated λ -chain 4548. Digest (2–3 mg) adjusted to pH 3.0 (see the Experimental section) was injected into a C-18 μ Bondapak reverse-phase column. The gradient system comprised solvent A (0.01 M-orthophosphoric acid adjusted to pH 3.0 with NaOH) and solvent B (methanol). A linear 0–70% gradient of solvent B was developed over 70 min at a flow rate of 1.5 ml/min and at room temperature. Fractions (0.75 ml) were collected every 30 s.

sequence determination, with the exception of fractions T₁, T₆ and T₇, which contained heterogeneous material. This allowed us to clearly determine that peptides in fractions T₂, T₃, T₄, T₅, T₈, T₁₁, T₁₂ and T₁₃ belonged to the V-region of λ -chain (I. Garcia & J.-C. Jaton, unpublished work). C-region peptides T₁, T₆ and T₇ were further purified by high-voltage paper electrophoresis at pH 6.5 or 3.5. The amino acid compositions of the tryptic peptides from the C-region of the λ -chain are given in Table 1. The hydrophobic peptides T₁₄ and T₁₅ were recovered in amounts not sufficient for total sequence analysis; a better yield of these fragments was obtained by h.p.l.c. analysis with the same initial aqueous solvent, but at pH 7.0 instead of 3.0, under the same elution conditions. Recoveries of all peptides appeared to be higher, e.g. 80% of the radioactivity in the cysteine-containing peptides was obtained at pH 7.0, instead of 45–50% at most at pH 3.0. However, at pH 7.0 the resolution of the hydrophilic peptides, T₁–T₆, was not as good as that illustrated in Fig. 1.

The fraction of the tryptic digest insoluble at pH 3.0 was freeze-dried, redissolved in 0.02 M-NH₃ and passed through a Sephadex G-50 column equilibrated in the same solvent. The major fraction eluting just after the void volume of the column was digested with α -chymotrypsin as outlined in Experimental section. The whole digest was separated by high-voltage paper electrophoresis at pH 3.5 and four major chymotryptic peptides were well resolved. Three of them were shown to belong to

the V-region (I. Garcia & J.-C. Jaton, unpublished work), whereas the other one, designated Ch₁, was shown to encompass the end of the V-region and the beginning of the C-region (Table 1).

In order to obtain some C-region overlapping peptides, the fully reduced and carboxamidomethylated λ -chain (0.3 μ mol) was digested with α -chymotrypsin and subjected to high-voltage paper electrophoresis at pH 3.5. From the digest, four well-separated peptides, Ch₁, Ch₂, Ch₃ and Ch₄, were shown to originate from the C-region, on the basis of their N- and C-terminal-sequence analysis. The determination of the amino acid sequence of the relevant C-region peptides is presented in Fig. 2. The sequence of all peptides (50–150 nmol) could be determined up to the C-terminal residue with the help of Polybrene, and the residues were unequivocally identified by h.p.l.c. Tryptic peptides were aligned by homology with the known murine and human C λ -region sequences (Dayhoff, 1978) and with the help of some overlapping chymotryptic peptides. The full amino acid sequence of rabbit λ -chain C-region is shown in Fig. 2.

Discussion

The complete amino acid sequence of the rabbit immunoglobulin λ L-chain C-region has been determined (Figs. 2 and 3). This chain was derived from the homogeneous anti-(type II pneumococcal polysaccharide) antibody fraction raised in Basilea rabbit

Table 1. *Amino acid composition of the constituent peptides of the C region of λ -chain 4548*

Values in parentheses are integral values confirmed by sequence analysis. T and Ch, tryptic and chymotryptic peptides respectively. Peptides T_{1a}, T_{1b}, T_{1c}, T₆, T₇ and Ch₁ were purified by high-voltage paper electrophoresis, all others by h.p.l.c. (see Fig. 1). Mobilities are expressed relative to arginine (= +1.0). Abbreviation used: CmCys, carboxymethylcysteine.

Amino acid	Peptide	Composition (residues/molecule of peptide)									
		T _{1a}	T _{1b}	T _{1c}	T ₆	T ₇	T ₉	T ₁₀	T ₁₄	T ₁₅	Ch ₁
Lysine		1.3 (1)	1.0 (1)	1.0 (1)	1.1 (1)		1.0 (1)	1.0 (1)	1.1 (1)		1.0 (1)
Histidine								2.5 (2)	0.8 (1)		
Arginine										0.8 (1)	
CmCys*						0.8 (1)		0.6 (1)		0.7 (1)	
Aspartic acid		2.1 (2)		2.1 (2)	1.0 (1)		3.1 (3)		0.9 (1)	1.0 (1)	
Threonine			1.4 (1)				2.7 (3)	2.8 (3)	0.7 (1)	0.9 (1)	3.3 (3)
Serine		0.7 (1)				1.9 (2)	1.6 (2)	2.3 (2)	1.8 (2)	0.8 (1)	2.5 (3)
Glutamic acid		1.0 (1)				1.0 (1)	2.2 (2)	3.8 (4)	1.3 (1)		2.7 (3)
Proline						0.9 (1)	1.1 (1)			1.0 (1)	3.7 (4)
Glycine							1.9 (2)	1.4 (1)			1.6 (1)
Alanine						2.3 (2)	1.2 (1)		3.5 (3)	1.3 (1)	1.2 (1)
Valine			1.0 (1)		0.8 (1)		2.5 (2)	2.6 (3)†		0.9 (1)	2.4 (3)‡
Isoleucine										0.8 (1)	1.1 (1)
Leucine						1.1 (1)			1.9 (2)	1.8 (2)	2.0 (2)
Tyrosine								0.8 (1)	0.7 (1)	0.7 (1)	
Phenylalanine									1.1 (1)	1.1 (1)	0.8 (1)
Tryptophan†					1†				†		
Total residues		5	3	3	4	8	17	18	15	13	23
Mobility at pH 6.5		+0.38	+0.52	0	+0.41	-0.55					

* Value not corrected for losses during hydrolysis.

† Measured with Ehrlich reagent (assumed to be one per molecule).

‡ Value after 22 h hydrolysis.

Fig. 2. *Amino acid sequence of the C region of rabbit λ -chain 4548*

For details, see the text. —, Residues identified by carboxypeptidases A + B. The one-letter notation for amino acid residues is that recommended in *J. Biol. Chem.* (1968) **243**, 3557–3559.

no. 4548 (Garcia & Jaton, 1979). Antibody IgG 4548 did not express L-chains of the b_4 , b_5 , b_6 and b_9 allotypes, as evidenced by the lack of reactivity with the corresponding anti-allotypic sera, but did express the C_1 allotypic specificity (Garcia & Jaton, 1979), which is considered to be a genetic marker characteristic of rabbit λ -chains (Mage *et al.*, 1973). In the sera of all hyperimmunized Basilea rabbits, antibody molecules whose L-chains belong to two distinct types were detected; the ratio of the two types was shown to be variable. Usually, the major antibody fraction contained λ L-chains, whereas a minor antibody fraction contained κ L-chains, which are structurally and antigenically distinct from the known b_4 , b_5 , b_6 and b_9 L-chain molecules (I. Garcia, D. Ch. Brandt, A. Benammar, P.-A. Cazenave & J.-C. Jaton, unpublished work). These different κ -chains in Basilea rabbits may correspond to the product of the Basilea gene controlled by the b locus, as recently suggested by Good *et al.* (1980). In the case of Basilea antiserum 4548, the homogeneous antibody fraction used in the present study contained L-chains of the λ type. The C-region amino acid sequence was established by analysis of the tryptic and some overlapping chymotryptic peptides derived from the λ -chain (Fig. 2). The few tryptic peptides for which no suitable overlap could be found have been unequivocally positioned in the sequence according to the high degree of homology with the C-region sequences of λ -chains from other species (Dayhoff, 1978) (Figs. 2 and 3). The precise delineation of the beginning of the λ C-region must await the characterization of the J gene, which encodes the peptide J that connects the V- to the C-region (Bernard *et al.*, 1978; Brack *et al.*, 1978). The first residue of the rabbit $C\lambda$ -region has thus been assigned by homology with λ -chains of other species, as shown in Fig. 3. The comparison of the sequence of the rabbit $C\lambda$ -region with the corresponding human, porcine and murine $C\lambda$ -sequences is shown in Fig. 3. Large interspecies similarities, as well as amino acid interchanges, are apparent among the various $C\lambda$ -regions; the degree of homology between rabbit $C\lambda$ -region and the $C\lambda$ -regions from human (Wikler *et al.*, 1967), porcine (Novotny *et al.*, 1977), murine (Appella, 1971; Dugan *et al.*, 1973) and guinea-pig chains (Brunhouse & Cebra, 1978) amounts to 72.6, 71, 72 and 73% respectively. The similarity between λ -chains of different species is much greater than that found between κ - and λ -chains of the same species, e.g. there is 34% homology between rabbit κb_9 chains (Farnsworth *et al.*, 1976) and rabbit λ -chain 4548, thus indicating a separate line of evolution of genes that encode κ - and λ -chains (Hood *et al.*, 1969). The high degree of homology between rabbit $C\lambda$ -domain and $C\lambda$ -domains of other species suggests that the point of divergence of rabbit λ -chain in phylogeny

	1	10	20	30	40	50																																															
Rabbit 4548	Q	P	A	V	T	P	S	S	E	E	L	K	D	N	K	A	T	L	V	C	L	I	S	D	F	Y	P	R	T	V	K	V	N	W	K	A	D	G	N	S	V	T	Q	G	V	D							
Human SH	—	K	A	A	—	T	—	—	—	—	Q	A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	A	—	T	—	A	—	—	S	S	P	—	K	A	—	E								
Pig	—	K	A	A	—	T	—	N	—	—	G	T	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	A	—	T	—	T	—	—	G	—	T	—	—	E										
λ 1 mouse MOPC 104E	—	K	S	S	—	T	—	T	—	E	—	T	E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	T	—	G	V	—	T	—	D	—	V	—	T	P	—	—	M	E								
λ 2 mouse MOPC 315	—	K	S	—	T	L	T	V	—	—	E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	N	—	S	—	G	S	—	T	—	A	—	N	—	T	P	I	—	B							
Rabbit 4548	T	T	Q	P	S	K	Q	S	N	N	K	Y	A	S	S	F	L	H	L	T	A	B	Q	W	(-)	K	S	Y	Q	S	V	T	C	Q	V	T	H	E	G	H	T	V	E	K	S	L	A	P	A	E	C	S	
Human SH	—	T	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	T	V	—	T
Pig	—	K	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
λ 1 mouse MOPC 104E	—	E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
λ 2 mouse MOPC 315	—	B	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Fig. 3. Comparison of λ L-chain C-regions of various species

λ L-chain C-region sequences from man, pig and mouse (λ 1 and λ 2) are from Wikler *et al.* (1967), Novotny *et al.* (1977), Appella (1971) and Dugan *et al.* (1973) respectively. (-), gap introduced to maximize the homology. The one-letter notation for amino acid residues is that recommended in *J. Biol. Chem.* (1968) **243**, 3557–3559.

occurred approximately at the time of murine and porcine λ -chain divergence (i.e. 75 million years ago; Barker *et al.*, 1978). It has previously been shown (Novotny & Franek, 1975) that, in the constant region of these chains, polypeptide segments that are folded back inside the domain (Poljak *et al.*, 1973) are phylogenetically highly conservative, whereas segments that are exposed to the surface of the molecule (i.e. portions 40–50 and 70–85 in Fig. 3) and marginally participate in folding architecture (Schiffer *et al.*, 1973) are much less conserved. Indeed, sequence differences between rabbit λ -chains and λ -chains of other species do occur precisely in two loops at the surface of the domain that correspond to the segments 40–50 and 70–85 according to the numbering system used in Fig. 3. These substitutions may possibly contribute to the allotypic or isotypic antigenic determinants of the λ -chain molecule (Mage *et al.*, 1973), as previously suggested in a similar fashion for the structural correlates of the V_H allotypes of the rabbit (Jaton *et al.*, 1976).

Very few structural data are yet available for the rabbit λ -chain system. Studies of λ -chains from Basilea rabbits therefore constitute an important approach to the problem of the genetic control of their biosynthesis. The data presented here should be extended to the full sequence determination of several λ L-chain C-regions in order to determine whether the extent of structural variation in the C λ -region resembles that of rabbit κ -chains. In the latter system, group-b allotypes associated with κ C-regions have been shown to be complex rather than simple on the basis of multiple amino acid substitutions throughout the κ C-region (Kindt, 1975; Emorine *et al.*, 1979). This raises the possibility that the corresponding alleles of the b locus may in fact be closely linked duplicated genes, regulated by a polymorphic-control mechanism (Farnsworth *et al.*, 1976). As to the λ -system, the same C-terminal tryptic peptide was identified in pooled λ L-chains from immunized and non-immunized Basilea animals (Garcia & Jaton, 1979). Thus the presence of distinct isotypes that would differ at the C-terminal end, as in the mouse (Blaser & Eisen, 1978), guinea pig (Brunhouse & Cebra, 1978) and man (Ein, 1968; Hess *et al.*, 1971), could not be detected in Basilea rabbits synthesizing λ -chains. However, fragmentary sequence data obtained with another λ -chain, 4431 (Garcia & Jaton, 1979), indicated two substitutions in C-region positions 46 and 47 when compared with λ -chain 4548 (Thr-Pro for Asn-Ser). Further, positions 84–85 are occupied by Ser-Val in λ -chain 4548, whereas the inverted sequence Val-Ser was reported in λ -chains from b_s -suppressed rabbits (Appella *et al.*, 1974) and in λ -chains from b_9/b_9 homozygous rabbits as well (Chersi, 1975). More interchanges

may become apparent when C λ -regions are fully sequenced. Thus the degree of polymorphism in λ -chains cannot be appreciated at the moment. Gene cloning and hybridization studies will be necessary to unravel how many C λ DNA copies are present in the Basilea genome.

As mentioned above, λ -chains present in normal domestic rabbits (Mage *et al.*, 1973), those synthesized as a compensatory balance of allotype suppression of κ -chains (Dubiski, 1967; Mage *et al.*, 1973), and those present in the variant rabbit strain Basilea (Garcia & Jaton, 1979) express C_7 and C_{21} allotypic markers. These allotypes were initially shown to segregate as simple Mendelian alleles, but matings in some other rabbit colonies produced no segregants (Gilman-Sachs *et al.*, 1969). Further observations led to the hypothesis that C_7 and C_{21} allotypes might be two or more distinct pseudo-allelic C λ -subtypes or even V λ -subgroups. The fact that the homogeneous λ -chain 4548 was recognized by both anti- C_7 and anti- C_{21} antisera (generously given by Dr. R. Mage, National Institutes of Health, Bethesda, MD, U.S.A.) makes the issue confusing at the present time. Preliminary attempts to relate the isotypic or allotypic specificities of homogeneous λ -chains of Basilea rabbits with the alleged C_7 and C_{21} specificities have been made with a panel of anti- λ -chain antisera.

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