Cloning the Arterial IgA Antibody Response during Acute Kawasaki Disease

Anne H. Rowley, Stanford T. Shulman, Francesca L. Garcia, Judith A. Guzman-Cotrill, Masaru Miura, Hannah L. Lee and Susan C. Baker

J Immunol 2005; 175:8386-8391; doi: 10.4049/jimmunol.175.12.8386
http://www.jimmunol.org/content/175/12/8386

References
This article cites 18 articles, 9 of which you can access for free at:
http://www.jimmunol.org/content/175/12/8386.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Cloning the Arterial IgA Antibody Response during Acute Kawasaki Disease

Anne H. Rowley,*† Stanford T. Shulman,* Francesca L. Garcia,* Judith A. Guzman-Cottrill,* Masaru Miura,* Hannah L. Lee,* and Susan C. Baker‡

Kawasaki disease (KD) is the most common acquired cardiac disease in children in developed nations. The etiology of KD is unknown but likely to be a ubiquitous microbial agent. Previously, we showed that oligoclonal IgA plasma cells infiltrate coronary arteries and other inflamed tissues in acute KD. We demonstrated that a synthetic Ab made using an α H chain sequence prevalent in acute KD arterial tissue detected Ag in acute KD coronary arteries, lung, and other inflamed tissues and that Ag localized to cytoplasmic inclusion bodies in the acute KD ciliated bronchial epithelium. In this study, we synthesized a panel of mAbs from α and κ chain sequences present in the KD arterial wall and tested the Abs for binding to acute KD tissues. We report that all of the synthetic mAbs that bind to acute KD tissues detect Ag in cytoplasmic inclusion bodies in the acute KD ciliated bronchial epithelium. Abs made from α sequences that were prevalent in KD arterial tissue showed stronger binding to acute KD tissues than Abs made from less prevalent sequences. These findings highlight the likely importance of the inclusion bodies in the etiopathogenesis of acute KD, confirm that the IgA Ab response in acute KD is Ag driven, and demonstrate the usefulness of cloning the Ab response in diseased tissues to identify disease-relevant Ags. The Journal of Immunology, 2005, 175: 8386–8391.

In this study, we sought to determine whether different KD synthetic Abs would detect Ags with diverse localization in KD tissues (to determine whether multiple different Ags, human and/or microbial, might be involved in pathogenesis) and to determine whether α sequences that were prevalent in acute KD arterial tissue would yield synthetic Abs that bind more strongly to KD Ag(s) than synthetic Abs made from α sequences that were less prevalent (to confirm an Ag-driven IgA response in acute KD). Therefore, we synthesized nine additional Abs from both prevalent and less prevalent IgA sequences in acute KD arterial tissue for a total of 13 KD synthetic mAbs, and we tested binding of the Abs to acute KD tissues. We show the first example of cloning the arterial Ab response in a human vasculitis to determine the potential antigenic targets of Abs produced by plasma cells infiltrating the inflamed vascular tissue.

Materials and Methods

Patients and specimens

Formalin-fixed, paraffin-embedded lung sections from KD patients 2, 7, and 8 (as described in Ref. 5) and patient 3 (as described in Ref. 6) with acute fatal KD were tested; the patients had coronary artery aneurysms at autopsy and died within 8 wk of illness onset. Abs A–J were also tested on autopsy and died within 8 wk of illness onset. Abs A–J were also tested on coronary artery and lymph node sections from acute KD fatalities (5, 6). The present study was approved by the Institutional Review Board of The Children’s Memorial Hospital.

Isolation of κ clones and DNA sequencing

The κ clones were isolated from a KD arterial cDNA library as described previously for κ clones (4), except a human κ probe (graciously provided by Dr. H.-M. Jäck, University of Erlangen, Erlangen, Germany) was used. Isolated phage clones were amplified using a primer in the pBluescript vector and a primer in the 5′ constant region of κ, as described previously for α clones (4) (primer sequence 5′→3′ CACTCTCCCCTGGT GAAGCCT). Clones that yielded a large enough product to contain a variable region (>300 bp) were sequenced as described previously (4), using a primer in the 5′ constant region of κ; primer sequence 5′→3′ AGCAGGGACACAAACAGGGCAGT.
Synthetic Ab production and labeling

KD synthetic Abs were derived from cDNA library clones containing α H chain sequences detailed previously (4) and either L chain 9-8 or L chain 6-11 (Table I) using γ1 and κ expression vectors (7). For Abs A–J, α H chain sequences were used with L chain sequence 9-8. Abs K, L, and M used H chains 2-2, C2, and 11-5, respectively, in combination with L chain 6-11 (Table II). Abs were synthesized and biotinylated as reported previously (5).

Immunohistochemistry experiments

Immunohistochemistry was performed as reported previously (5), using 10–50 μg/ml biotinylated synthetic Ab and a Vectastain Elite ABC kit. The Journal of Immunology
Incubated with biotinylated synthetic Ab at both 0.5 and 5 g/ml. Wells coated with 100 ng of human IgG (from human serum; Sigma-Aldrich), or purified human IgM (myeloma; Jackson ImmunoResearch Laboratories), IgG1 (from human serum; Jackson ImmunoResearch Laboratories), IgG1 κ (myeloma; Sigma-Aldrich), IgG2 κ (myeloma; Sigma-Aldrich), IgG1 λ (myeloma; Sigma-Aldrich), or purified human λ, L chains (myeloma; Sigma-Aldrich) and incubated with biotinylated synthetic Ab at both 0.5 and 5 μg/ml. Wells were then incubated with avidin-HRP (Pierce) and Super Signal West Pico chemiluminescence substrate (Pierce) according to the manufacturer’s instructions.

**Immunoprecipitation**

Lysates from acute KD spleen tissue that was positive by immunohistochemistry using synthetic Abs were subjected to immunoprecipitation with synthetic Abs A–F and J using the Seize X immunoprecipitation kit (Pierce) according to the manufacturer’s instructions, and Western blots of the immunoprecipitated material were probed with biotinylated synthetic Abs A–F and J using the Pico chemiluminescence substrate (Pierce) according to the manufacturer’s instructions.

**Enzyme-linked immunosorbent assays**

To determine whether synthetic Abs were anti-Ig Abs, ELISA wells were coated with 100 ng of human IgG (from human serum; Sigma-Aldrich), IgM (myeloma; Jackson ImmunoResearch Laboratories), IgA (from human serum; Jackson ImmunoResearch Laboratories), IgG1 κ (myeloma; Sigma-Aldrich), IgG2 κ (myeloma; Sigma-Aldrich), IgG1 λ (myeloma; Sigma-Aldrich), or purified human κ L chains (myeloma; Sigma-Aldrich) and incubated with biotinylated synthetic Ab at both 0.5 and 5 μg/ml. Wells were then incubated with avidin-HRP (Pierce) and Turbo TMB (Pierce) according to the manufacturer’s instructions, and absorbance at 450 nm was recorded. Positive control wells were incubated with goat anti-human IgG, IgM, and IgA (H and L chains) (Pierce) at 0.5 μg/ml instead of synthetic Ab.

**Results**

We synthesized 13 mAbs using prevalent and less prevalent κ H chain sequences reported previously (4) in combination with one of two κ L chain sequences and tested their binding to acute KD tissues.

### Analysis of κ L chains

Sixty-one κ L chain sequences were sequenced; these κ sequences were more polyclonal than the κ H chains and are shown in Table I; potential clonally related sequences appear in italic type, as defined by sequences with the same CDR3 length and JK usage with few amino acid differences. The polyclonal nature of the κ sequences is likely explained by the presence of IgM Abs in addition to IgA Abs in the arterial tissue, as described previously (2), potentially increasing the diversity of the L chain sequences in the tissue. The κ sequences 9-8 and 6-11 were selected for Ab production because there appeared to be multiple potentially clonally related sequences corresponding to these variable chains in the tissue; these sequences are indicated in bold type. Although apparent selection of 11 aa CDR3 domains was reported previously among V κ III sequences in the peripheral blood of children with acute KD (8), we found only one 11 aa CDR3 domain among 23 V κ III sequences in the KD arterial wall.

### Expression of KD synthetic Abs

Successful Ab production was achieved for three of the five most prevalent κ H chain sequences in KD arterial tissue (2-2, C2, and 11-5) (Table II). We hypothesized that these Abs would be more likely to show strong binding to acute KD tissues than Abs containing less prevalent κ H chain sequences. To test this hypothesis, we also made Abs using seven κ H chain sequences that were represented only once each in the original group of 44 κ sequences (Table II). Singly represented full-length clones were selected at random for Ab production, although H chain clone E1 was chosen for Ab production because the sequence was identified in a limited number of clones in both spleen and arterial tissue from an acute KD patient (4).

Ab production was unsuccessful for the other two prevalent κ H chains. H chain E2, the most prevalent sequence identified in acute KD arterial tissue, was used in multiple transfection experiments with L chains 9-8 and 6-11, but yields of Ab in tissue culture supernatants were too low to allow for successful production, possibly because the L chains did not interact appropriately with the H chain. The three H chain clones obtained from the VH7 group I family were not full length; screening the original cDNA library for a full-length VH7 group I clone was not successful (data not shown).

### Immunohistochemistry results

Abs A, J, K, and M were synthesized from prevalent H chains 2-2 and 11-5 and showed strong binding to acute KD tissues; Abs D and L, made from prevalent H chain C2, did not bind. Of all the Abs synthesized, Abs J and M showed the strongest binding to acute KD tissues. None of seven Abs made from less prevalent sequences found only once each in the original 44 κ sequences as detailed previously (4) showed strong binding to acute KD tissues. Four of the seven less prevalent Abs showed weak binding, and three showed no binding. Immunohistochemistry results using Abs A–D and J on the acute KD bronchial epithelium were demonstrated in our previous reports (5, 6). Results using Abs F, I, and J on the acute KD bronchial epithelium are shown in Fig. 1, and results using Abs K and M are shown in Fig. 2. None of the Abs showed binding to the control bronchial epithelium (results using Ab J are shown in Fig. 1D).

Notably, all Abs that detected Ag in acute KD tissues showed the same pattern of binding as Ab A, to spheroid inclusion bodies in the acute KD ciliated bronchial epithelium and to a subset of macrophages in the inflamed acute KD coronary artery and lymph node (5). Ab E stained KD Ag and a subset of plasma cells in KD and control tissues; none of the other Abs stained plasma cells.

Blocking experiments were performed by incubation of duplicate tissue sections with biotinylated synthetic Ab A and with unlabeled Ab A, followed by biotinylated Ab J. A similar experiment was performed using Ab J as the blocking Ab. These results were somewhat difficult to interpret because duplicate sequential sections incubated with the same synthetic Ab can show somewhat variable intensity of staining. Neither A nor J completely blocked binding of the other Ab, but a partial blocking effect could not be

### Table II. KD Synthetic Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Staining of Acute KD Tissues</th>
<th>Abundance of κ H Chain (n = 44) (Ref. 4)</th>
<th>H Chain</th>
<th>L Chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Strong</td>
<td>2</td>
<td>2–2</td>
<td>9–8</td>
</tr>
<tr>
<td>J</td>
<td>Strong</td>
<td>3</td>
<td>11-5</td>
<td>9–8</td>
</tr>
<tr>
<td>K</td>
<td>Strong</td>
<td>2</td>
<td>2–2</td>
<td>6–11</td>
</tr>
<tr>
<td>M</td>
<td>Strong</td>
<td>3</td>
<td>1-11</td>
<td>6–11</td>
</tr>
<tr>
<td>B</td>
<td>Weak</td>
<td>1</td>
<td>2–4</td>
<td>9–8</td>
</tr>
<tr>
<td>C</td>
<td>Weak</td>
<td>1</td>
<td>E1</td>
<td>9–8</td>
</tr>
<tr>
<td>E</td>
<td>Weak</td>
<td>1</td>
<td>2–1</td>
<td>9–8</td>
</tr>
<tr>
<td>F</td>
<td>Weak</td>
<td>1</td>
<td>H1</td>
<td>9–8</td>
</tr>
<tr>
<td>D</td>
<td>None</td>
<td>2</td>
<td>C2</td>
<td>9–8</td>
</tr>
<tr>
<td>G</td>
<td>None</td>
<td>1</td>
<td>C3</td>
<td>9–8</td>
</tr>
<tr>
<td>H</td>
<td>None</td>
<td>1</td>
<td>C6</td>
<td>9–8</td>
</tr>
<tr>
<td>I</td>
<td>None</td>
<td>1</td>
<td>4–2</td>
<td>9–8</td>
</tr>
<tr>
<td>L</td>
<td>None</td>
<td>2</td>
<td>C2</td>
<td>6–11</td>
</tr>
</tbody>
</table>
excluded. Because of the limited quantity of tissue samples available from patients with acute fatal KD, blocking experiments could not be performed with every synthetic Ab combination.

**Substituting the L chain partner**

Substituting L chain 9-8 for L chain 6-11 had minor effects on Ag binding when used in combination with H chains 2-2, C2, and 11-5. Abs D and L both showed negative results, and A and K both showed strong binding. J and M both showed strong binding; J appeared to have overall stronger binding characteristics than M, but this was subtle.

**Immunoprecipitation**

All Abs gave negative results, with the exception of Ab E. This Ab immunoprecipitated a 22 kDa protein that was identified as Ig κ L.
chain by MALDI-mass spectrometry (performed at the core protein facility at Columbia University, New York, NY; data not shown).

**Enzyme-linked immunosorbent assay**

Because synthetic Ab E showed binding to a subset of plasma cells in KD and control lung tissue as well as to KD Ag in the acute KD bronchial epithelium but not control bronchial epithelium, and because it appeared to bind to $\kappa$ L chain in immunoprecipitation experiments, it was tested in ELISAs to determine whether it was an anti-$\kappa$ Ab. Ab E gave positive results in ELISAs using polyclonal IgG and IgA from human serum and negative results using myeloma Abs IgM $\kappa$, IgGl $\kappa$, and IgG1 $\kappa$. It gave weakly positive results using myeloma IgG2 $\kappa$ and purified $\kappa$ chains. Abs A, D, and H, which show binding to KD Ag but not to plasma cells, were also tested and did not demonstrate any binding to any of the above Iggs. Ab E did not react with Ab A when A was used to coat ELISA wells. From these results, we concluded that Ab E binds to a subset of $\kappa$ L chains and therefore more likely interacts with the variable region of $\kappa$ than the constant region. Because Ab E binds to both a subset of $\kappa$ Abs and to KD Ag, it most likely reacts with immune complexes, probably directed at a conformational epitope of an Ag-Ab complex in which KD Ag interacts with the $\kappa$ L chain.

**Discussion**

In this study, we cloned the IgA immune response using Ig $\alpha$ sequences that we identified previously in the KD arterial wall (4) and obtained the somewhat unexpected result that the synthetic Abs made from these sequences that bind to acute KD tissues all appear to bind to cytoplasmic inclusion bodies in the acute KD ciliated bronchial epithelium. This result emphasizes the importance of these inclusions in disease etiopathogenesis. We also found that Abs made from two prevalent $\alpha$ H chains, 2-2 and 11-5, bind more strongly to acute KD tissues than do Abs made from less prevalent $\alpha$ chains, as would be expected in an Ag-driven response.

Although we did not originally anticipate that all synthetic Abs that detected Ags in acute KD tissues would show the same pattern of binding, this result is consistent with the detection of a microbial pathogen. Abs to certain microbial Ags can dominate the immune response. For example, Burgoon et al. (9, 10) prepared synthetic Abs from overrepresented IgG sequences in the brains of patients with subacute sclerosing panencephalitis (SSPE) and demonstrated that all of the synthetic Abs that bound to SSPE brain tissue reacted with measles virus nucleocapsid.

Because the H chain is more important than the L chain for Ag binding (11, 12), we performed these studies with $\alpha$ H chain sequences in random combination with one of two $\kappa$ L chains. However, on occasion, changing an L chain partner can drastically affect Ag binding (13); it is possible that H chain C2 (Abs D and L) requires an L chain partner other than 9-8 or 6-11 (either a $\kappa$ or a $\lambda$ L chain) to assume the optimal conformation for Ag binding. Moreover, the affinity of a synthetic Ab for its Ag can be reduced significantly with the wrong L chain partner, even if some binding is preserved. Burgoon et al. (9) prepared synthetic Abs from SSPE patients using random combinations of L and H chains, similar to our study. They found that these Abs all detected measles virus nucleocapsid Ag in infected cells but did not bind to Ag in immunoblots or immunoprecipitations (9). However, when they isolated single plasma cells and performed RT-PCR to determine correct L and H chain partners, Abs made from the correct pairing detected measles virus nucleocapsid Ag in infected cells and also bound to Ag in immunoblot assays (10). In limited Western blot and immunoprecipitation studies on acute KD spleen tissue that gave positive results by immunohistochemistry, and more extensive screening of a cDNA expression library made from this tissue (data not shown), we have not detected Ag using our synthetic Abs (except for $\kappa$ L chain as described above for Ab E). This interesting parallel between our work and the work of Burgoon et al. (9, 10) of measles in patients with SSPE, in which synthetic Abs made from random pairing of L and H chains detect Ag in cells but not in immunoblots and immunoprecipitations, has led us to begin studies to perform RT-PCR on single isolated plasma cells in acute KD arterial tissue, to obtain the correct L and H chain pairings, so that we can test these new Abs in immunohistochemistry, immunoblots, immunoprecipitation, and cDNA library screening experiments.

Interestingly, one of the 13 synthetic Abs, Ab E, appeared to bind to both KD Ag and to a subset of Ig $\kappa$ molecules. It is possible that the binding features of this Ab were the result of an incorrect L chain (9-8) used with H chain 2-1 and that pairing H chain 2-1 with the correct L chain would have resulted in an Ab that only bound to KD Ag. However, anti-Abs responses have been linked to infectious agents, particularly those that expose highly ordered repetitive antigenic epitopes and form immune complexes in vivo (14). It has been postulated that anti-idiotypic Abs formed during infection may enhance the immunopathology of disease (14).

The panel of monoclonal KD Abs synthesized in this study have been useful in demonstrating that the IgA immune response in the acute KD arterial wall is Ag driven and that Ag(s) localized to cytoplasmic inclusion bodies in the acute KD ciliated bronchial epithelium appear to be a primary target of the IgA immune response in acute KD, emphasizing the importance of identifying these Ags. The extreme scarcity of fresh or frozen tissue samples from acute KD patients has slowed progress considerably in identifying the Ag(s) present in the inclusions. These inclusion bodies can be visualized using a variety of light microscopy stains and are revealed by transmission electron microscopy to be regular, homogeneous inclusion bodies that resemble aggregates of viral proteins and nucleic acids, such as nucleocapsid aggregates of the Paramyxoviridae (6). We cannot find a precedent for a human Ag to localize to intracellular inclusion bodies during an acute human illness, but numerous infectious agents (mostly viral) can result in inclusion body formation in acutely infected cells (15–19).

Identification of the Ags important in KD pathogenesis is critical to understanding the etiology and pathogenesis of this potentially fatal illness of childhood. In vitro synthesis of Abs made by plasma cells that infiltrate diseased tissue and their use as reagents to detect target Ags, as in our studies of KD and in those of Burgoon et al. (9, 10) to study SSPE, may also prove useful in determining the pathogenesis of other infectious and inflammatory disorders, including other vascular diseases in which plasma cell infiltration into vascular tissues is observed.

**Disclosures**

The authors have no financial conflict of interest.

**References**


