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Research Article

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A Natural Autoantibody Is Encoded by Germline Heavy and Lambda Light Chain Variable Region Genes without Somatic Mutation

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Abstract

While nonmutated germline variable region (V) genes have been found to encode heavy or light chains of various human autoantibodies, the use of germline V genes by both chains of a given autoantibody has not been documented. Recently, we reported that the heavy chain V gene (designated Humha346) of the Kim4.6 anti-DNA antibody is identical to a germline VH gene, 1.9III. To investigate whether this autoantibody was entirely germline encoded, we searched for the germline counterpart to the Kim4.6 Vλ segment (designated Humla146) and isolated a VλI gene designated Humlv117, which was identical to Humla146. Together with the sequence identity of the Kim4.6/Humha346 and 1.9III VH genes, the current data provide the first direct proof that an autoantibody can be encoded entirely by germline V genes without any somatic change. In addition, Humlv117 is the first VλI germline gene that has been isolated, and is highly homologous to the Vλ genes expressed in two lymphomas. Thus, this VλI gene should provide a useful tool for investigating the expression of the human Vλ gene repertoire, particularly with regard to autoimmune and/or lymphoproliferative diseases.

Introduction

During the past few years, there has been major progress in the elucidation of the genetic mechanisms for antibody production as they relate to many antigen-specific immune responses (1–3). Until recently, however, little information has been available with regard to the genetic basis of specific autoimmune responses, and in particular, the contribution of variable region (V)[1] genes and somatic mutation to the generation of such responses. From studies in many laboratories it has been shown that there is frequent sharing of crossreactive idiotypes among human autoantibodies of given specificities, implying that they use the same or similar germline V genes with little somatic change (4–6). This interpretation is supported by the finding of extensive similarity among the heavy and light chain V regions of idiotypically related monoclonal autoantibodies (7, 8), and more recently by data showing complete identity between a germline Vκ gene (Humkv325) and four human rheumatoid factor light chains (9, 10). However, whether antibodies reactive with self-antigens can be encoded by heavy and light chain germline V genes that are both unaltered by somatic mutation remains unclear.

Among the various human autoantibodies, anti-DNA antibodies have been a particular object of investigation, originally in the context of their strong association with systemic lupus erythematosus (SLE) and, more recently, with regard to their occurrence in apparently healthy individuals (5). To investigate the genetic origins of this autoimmune response, we undertook studies of V gene utilization in "natural" anti-DNA antibodies derived from nonautoimmune subjects. Included among these was an IgMλ anti-DNA monoclonal antibody (designated Kim4.6), derived from nonautoimmune tonsillar lymphoid cells and reactive with both single- and double-stranded DNA, synthetic polynucleotides, RNA and cardiolipin (11). As previously reported, this autoantibody uses a heavy chain V (VH) gene identical in sequence to a germline VH gene, 1.9III (12, 13). Although the sequence of the Kim4.6 lambda light chain V (Vλ) gene (Humla146) was also determined, its relationship to the germline gene repertoire could not be assessed since very few data were available with regard to human germline Vλ gene sequences. Similarly, while the use of nonmutated germline VH genes has been reported for several human monoclonal autoantibodies derived from SLE patients, as for the Kim4.6 antibody, the concomitant expression of nonmutated light chain V genes in these antibodies has not been documented (14–16). Accordingly, the possibility remains that somatic change in the V gene of one chain of an antibody is necessary for the generation of self-reactivity. To address this issue directly, we searched for the germline counterpart of the Humla146 lambda light chain. In this paper, we report on the isolation of a novel human germline Vλ gene, designated Humlv117, and show that its nucleotide sequence is identical to that of Humla146. These findings provide formal proof that human autoantibodies can be encoded by germline V genes without somatic mutation. In addition, Humlv117 is highly homologous to the Vλ gene sequences expressed in two lymphomas. Thus, this newly isolated VλI gene should provide a very useful tool for investigating the pattern of Vλ gene utilization and diversification in autoimmune responses and other immunological contexts.

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1. *Abbreviations used in this paper:* la, a rearranged lambda V gene; lv, a germline lambda V gene; RF, rheumatoid factor; SSC, standard saline citrate; V, variable region; VH, heavy chain V gene; Vκ, kappa light chain V gene; Vλ, lambda light chain V gene.

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Methods

Library, probes, and screening. The human genomic library was kindly provided by Dr. Wen-Hwa Lee (University of California at San Diego, La Jolla, CA), and was constructed by cloning partially digested DNA from the Y79 retinoblastoma cell line into EMBL-3 (17). One million recombinant clones were screened with either a 1.05-kb SmaI fragment (containing the VJ and the 5′ portion of the Cλ regions) or a 0.8-kb SmaI-MspI fragment (containing most of the Vλ region) of the Kim4.6/Humla146 cDNA clone. Hybridizations were done in 2× standard saline citrate (SSC) (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) at 65°C, followed by washing twice in 1× SSC at 65°C (18).

Plaques positive for hybridization with either probe were purified and analyzed by restriction mapping. Appropriate fragments were subcloned into pUC18 and pUC19 and double-stranded sequencing was performed by dideoxy chain termination using ^{35}S-dATP (Amersham Corp., Arlington Heights, IL) (19). Computer programs of the University of Wisconsin Genetics Computer Group were used to analyze the sequence data (20).

Results and Discussion

To isolate the germline counterpart to the Humla146 gene encoding the Kim4.6 lambda light chain, 10^6 recombinant phage plaques of a human genomic library were screened with subfragments of the Humla146 cDNA clone. Among the 19 positive clones identified, the 5 displaying the strongest hybridization signals were further purified and subcloned into pUC vectors for nucleotide sequence analysis. The sequence of the VλI gene contained in one of these isolates (designated Humlv117) is shown in Fig. 1 along with the sequences of the Kim4.6/Humla146 and the closely related Vλ genes expressed in two lambda-secreting lymphomas, T2/C5 and BL-2 (21, 22). As indicated in Fig. 1, the germline V segment, designated Humlv117, displays complete sequence identity to the Humla146 gene over a stretch of 348 bp beginning 54 bp upstream of the codon for the first amino acid residue.

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                                                    lyS  W  A  Q  S      2
                                                     .  **  .  1  .
lv117  TCTAGACCAAGAATCACCGTGTCTGTCTCTCCTGCTTCCAGGGTCCTGGGCCCAGTCT     6
la146  ------------------------------------------------------------
T2/C5  ..........................................deleted/-----------
BL-2   ..........................................deleted/-----------

       V  L  T  Q  P  P  S  V  S  A  A  P  G  Q  K  V  T  I  S  C     22
lv117  GTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATCTCCTGC    66
la146  ------------------------------------------------------------
T2/C5  ------------------------------------------------------------
BL-2   ------------------------------------------------------------

       S  G  S  S  S  N  I  G  N  N  Y  V  S  W  Y  Q  Q  L  P  G     42
      23_____________CDR1_____________35  .  .  .  .  .  .  .  .
lv117  TCTGGAAGCAGCTCCAACATTGGGAATAATTATGTATCCTGGTACCAGCAGCTCCCAGGA    126
la146  ------------------------------------------------------------
T2/C5  ------------------------------------------------------------
BL-2   --------------------------G------------------A---G---------

       T  A  P  K  L  L  I  Y  E  N  N  K  R  P  S  G  I  P  D  R     62
                .  .  51_____CDR2_____57  .  .  .  .  .  .  .  .
lv117  ACAGCCCCCAAACTCCTCATCTATGAAAATAATAAGCGACCCTCAGGGATTCCTGACCGA    186
la146  ------------------------------------------------------------
T2/C5  --------------------T-----C-------------------------------
BL-2   --------------------T-----C--------A----------------------

       F  S  G  S  K  S  G  T  S  A  T  L  G  I  T  G  L  Q  T  G     82
lv117  TTCTCTGGCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGACTCCAGACTGGG    246
la146  ------------------------------------------------------------
T2/C5  ------------------------------------------------------------
BL-2   ------------------------------------------------------------

       D  E  A  D  Y  Y  C  G  T  W  D  S  S  L  S  A                98
                .  .  .90_____CDR3_____98  .*7-mer*  .
lv117  GACGAGGCCGATTATTACTGCGGGAACATGGGATAGCAGCCTGAGTGCTGGCACAGTGGTC    306
la146  ----------------------------------------------/rearranged
T2/C5  ----------------------------------------------/rearranged
BL-2   ----------------------------A---A----------G-/rearranged

                .  .  .**9-mer**.  .  .  .  .  .  .  .  .  .  .  .
lv117  CAGCCCAATGGGGAACTGAGACAAGAACCCCCTTCTTCCTCCCCCAGGAGGGTGAGTGCC    366
lv117  GCCAGCTGCTGCTCACGCCTGACCTGTAGCTTCTGCTGCTGCAG                  410
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Figure 1. Genomic structure of the Humlv117 gene. The sequences of the VλI genes rearranged in the Kim4.6 natural hybridoma, a large cell lymphoma line, and a Burkitt lymphoma (Humla146, T2/C5, and BL-2, respectively) are shown for comparison. The deduced amino acid sequence for Humlv117 is shown in the top line. All sequences are aligned for maximum homology and gaps are indicated by dots, nucleotide identity by dashes. The conserved sequences for splicing and rearrangement, including AG, heptamer and nonamer, are marked. CDR, complementarity determining region.

These results demonstrate unequivocally that Humlv117 represents the germline gene corresponding to Humla146 and thus indicate the use of a nonmutated germline V segment by the Kim4.6 anti-DNA antibody. Taken together with the previous finding of sequence identity between the Kim4.6 VH gene (Humha346) and the 1.9III germline VH segment (13), the current results provide the first direct proof that a human "natural" autoantibody can be entirely encoded by germline V genes unaltered by somatic mutation. The sequence identity between Humla146 and Humlv117, Vλ segments that have been isolated from two unrelated individuals, also provides evidence that autoantibody-associated Ig V genes are conserved within the outbred human population (14–16). The apparent evolutionary pressure for preservation of V genes used in a natural autoantibody implies that autoreactivity plays a physiologic role within the immune system, possibly by influencing the development of the B lymphocyte repertoire.

Previous studies of Vκ gene usage among human autoantibodies revealed that the Humkv325 amino acid sequence was identical to the Vκ sequences of four rheumatoid factors and one antibody directed against the intermediate filament, and differed from the Vκ sequence of an anti-low density lipoprotein antibody by only one amino acid residue (9, 10). In addition, expression of Humkv325 was found in 20% of human chronic lymphocytic leukemias, a tumor arising from the autoreactive CD5 B cell subset (23, 24). These data raise the possibility that other autoantibody-associated V genes, such as Humlv117, might also be used preferentially by paraproteins that exhibit autoreactivity, and be expressed frequently in malignant B cells. In this regard, it is noteworthy that Humlv117 is highly homologous to the single V nucleotide sequence that has been reported for VλI-secreting Burkitt lymphomas as well as a Vλ segment recently isolated from a diffuse large cell lymphoma line (Figs. 1 and 2) (21, 22). In addition, Humlv117 displays > 90% amino acid sequence homology with five previously published VλI paraproteins (Fig. 2) (25). 11 other paraproteins assigned to the λI subgroup share a lesser degree of homology with Humlv117 and are likely to be encoded by other germline genes and represent a distinct VλI sub-subgroup as suggested previously (26). The only other human germline Vλ gene that has been previously cloned and sequenced (termed 4A) displayed < 50% amino acid sequence similarity to any of the lambda light chain sequences from all six Vλ subgroups defined by amino acid sequence comparisons (25, 27). Isolation and characterization of additional human germline Vλ genes, as well as Vλ sequences expressed by human autoantibodies and in malignant B cells, should provide important insights into the genetic diversity of the human Vλ gene repertoire and the pattern of Vλ gene utilization in autoimmune and/or lymphoproliferative diseases.

Our finding that the Kim4.6 anti-DNA antibody is germline encoded is consistent with the view that natural autoanti-

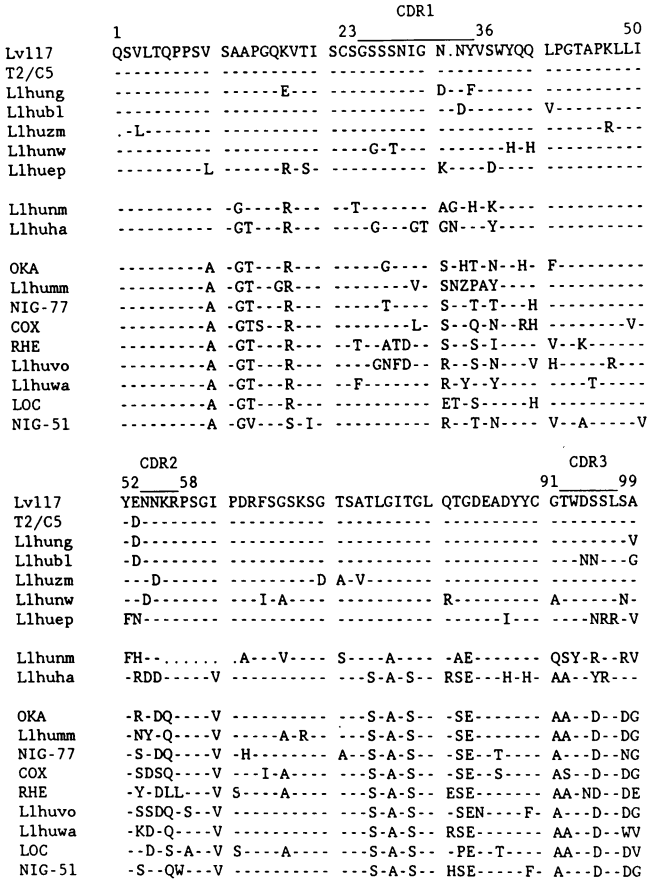

Figure 2. Comparison of the deduced amino acid sequence of Humlv117 and all available λI light chains. Listed to the left are the names of the amino acid (or deduced amino acid) sequences. The light chains beginning with "Llhu" are from the National Biomedical Research Foundation protein database (release 19, December 1988). "Llhuzm" is a temporary designation for the light chain designated A29700 in the NBRF database. Except for T2/C5, a recently reported Vλ gene (22), the remaining sequences are from "Sequences of proteins of immunological interest" (25). We also searched the GenBank database (release 58, December 1988) and the EMBL database (release 15, April 1988) and found no additional VλI sequences. The dashed lines represent amino acid identity to Humlv117. The sequences are aligned to maximize homology. The seven V region sequences shown in the top grouping display > 90% homology with each other and lesser homology with the sequences included in the lower two groupings.

bodies, which are generally of the IgM isotype, polyspecific, and idiotypically crossreactive, are representative of early ontogenic or primary immune responses (5, 28, 29). Similarly, the VH gene of another human anti-DNA antibody (18/2) is identical in sequence with a germline VH gene, VH26, and the VH gene encoding an anti-Sm antibody (4B4) displays sequence identity with that of a fetal liver cDNA (20P1/M26) derived from a different individual, suggesting the latter two sequences also represent nonmutated forms of a germline gene (14–16, 30). By contrast, the properties ascribed to "pathogenic" autoantibodies, IgG isotype, fine specificity, and high affinity, are characteristic of the somatically mutated antibodies associated with secondary immune responses (6, 31, 32). Extensive somatic diversification has, in fact, been found among the IgG autoantibodies of autoimmune mice and appears to correlate with the development of high affinity autoantibodies (31, 32). Taken together, these results suggest that autoimmune responses use the same molecular mechanisms of diversification found in non-self antigen-driven responses, and that their utilization of germline or somatically mutated V genes is likely to reflect the developmental stage and immunologic context in which the response originates.

Finally, it is noteworthy that two previously sequenced autoantibodies, the 18/2 anti-DNA and 4B4 anti-Sm antibodies, use germline VH genes identical with VH genes that appear to be preferentially expressed in the fetal pre-B cell repertoire (14, 16, 30). Similarly, the VH gene encoding the Kim4.6 autoantibody belongs to the restricted set of VH genes expressed among fetal liver B cells (12, 30). It therefore appears likely that autoreactive antibodies are relevant to the development and maintenance of the normal immune repertoire, and it is plausible that an abnormal expansion and diversification of the natural preimmune repertoire may be related to the appearance of "pathogenic" autoantibodies associated with autoimmune disease.

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References

1. Tonegawa, S. 1983. Somatic generation of antibody diversity. *Nature (Lond.).* 302:575–581.

2. Rajewsky, K., I. Forster, and A. Cumano. 1987. Evolutionary and somatic selection of the antibody repertoire in the mouse. *Science (Wash. DC).* 238:1088–1094.

3. Manser, T., L. J. Wysocki, M. N. Margolies, and M. L. Gefter. 1987. Evolution of antibody variable region structure during the immune response. *Immunol. Rev.* 96:141–162.

4. Williams, R. C., H. G. Kunkel, and J. D. Capra. 1968. Antigenic specificities related to the cold agglutinin activity of gamma M globulins. *Science (Wash. DC).* 161:379–381.

5. Zouali, M., B. D. Stollar, and R. S. Schwartz. 1988. Origin and diversification of anti-DNA antibodies. *Immunol. Rev.* 105:137–159.

6. Davidson, A., A. Livneh, A. Manheimer-Lory, R. Shefner, J. B. Katz, K. L. Sewell, and B. Diamond. 1988. Idiotypic analyses of anti-DNA antibodies in systemic lupus and monoclonal gammopathy. *Ann. Inst. Pasteur Immunol.* 139:645–650.

7. Capra, J. D., and D. G. Klapper. 1976. Complete amino acid sequence of the variable domains of two human IgM anti-gamma globulins (Lay/Pom) with shared idiotypic specificities. *Scand. J. Immunol.* 5:677–684.

8. Andrews, D. W., and J. D. Capra. 1981. Complete amino acid sequence of variable domains from two monoclonal human anti-gamma globulins of the Wa cross-idiotypic group: suggestion that the J segments are involved in the structural correlate of the idiotype. *Proc. Natl. Acad. Sci. USA.* 78:3799–3803.

9. Chen, P. P., K. Albrandt, T. J. Kipps, V. Radoux, F. T. Liu, and D. A. Carson. 1987. Isolation and characterization of human VκIII germline genes: implications for the molecular basis of human VκIII light chain diversity. *J. Immunol.* 139:1727–1733.

10. Chen, P. P., S. Fong, F. Goni, G. J. Silverman, R. I. Fox, M. F. Liu, B. Frangione, and D. A. Carson. 1988. Cross-reacting idiotypes on cryoprecipitating rheumatoid factor. *Springer Semin. Immunopathol.* 10:35–55.

11. Cairns, E., J. Block, and D. A. Bell. 1984. Anti-DNA autoantibody-producing hybridomas of normal human lymphoid cell origin. *J. Clin. Invest.* 74:880–887.

12. Cairns, E., P. C. Kwong, V. Misener, P. Ip, D. A. Bell, and K. A. Siminovitch. 1989. Analysis of variable region genes encoding a human anti-DNA antibody of normal origin: implications for the molecular basis of human autoimmune responses. *J. Immunol.* 143:685–691.

13. Berman, J. E., S. J. Mellis, R. Pollock, C. L. Smith, H. Suh, B. Heinke, C. Kowal, U. Surti, L. Chess, C. R. Cantor, and F. W. Alt. 1988. Content and organization of the human Ig VH locus: definition of three new VH families and linkage to the Ig CH locus. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:727–738.

14. Dersimonian, H., R. S. Schwartz, K. J. Barrett, and B. D. Stollar. 1987. Relationship of human variable region heavy chain germ-line genes to genes encoding anti-DNA autoantibodies. *J. Immunol.* 139:2496–2501.

15. Chen, P. P., M.-F. Liu, S. Sinha, and D. A. Carson. 1988. A 16/6 idiotype positive anti-DNA antibody is encoded by a conserved VH gene with no somatic mutation. *Arthritis Rheum.* 31:1429–1431.

16. Sanz, I., H. Dang, M. Takei, N. Talal, and J. D. Capra. 1989. VH sequence of a human anti-Sm autoantibody. Evidence that autoantibodies can be unmutated copies of germline genes. *J. Immunol.* 142:883–887.

17. Lee, W. H., R. Bookstein, F. Hong, L.-J. Young, J.-Y. Shew, and Y.-H. P. Lee. 1987. Human retinoblastoma susceptibility gene: cloning, identification and sequence. *Science (Wash. DC).* 235:1394–1399.

18. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *In* Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 387–389.

19. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

20. Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387–395.

21. Tsujimoto, Y., and C. M. Croce. 1984. Molecular cloning of a human immunoglobulin chain variable sequence. *Nucleic Acids Res.* 12:8407–8414.

22. Berinstein, N., S. Levy, and R. Levy. 1989. Activation of an excluded immunoglobulin allele in a human B lymphoma cell line. *Science (Wash. DC).* 244:337–339.

23. Preud'homme, J. L., and M. Seligmann. 1972. Anti-human immunoglobulin G activity of membrane-bound monoclonal immunoglobulin M in lymphoproliferative disorders. *Proc. Natl. Acad. Sci. USA.* 69:2132–2135.

24. Kipps, T. J., E. Tomhave, P. P. Chen, and D. A. Carson. 1988. Autoantibody-associated kappa light chain variable region gene expressed in chronic lymphocytic leukemia with little or no somatic mutation. Implications for etiology and immunotherapy. *J. Exp. Med.* 167:840–852.

25. Kabat, E. A., T. T. Wu, M. Reid-Miller, H. Perry, and K. S. Gottesman. 1987. Sequences of proteins of immunological interest. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD. 63–77.

26. Kametani, F., T. Takayasu, S. Suzuki, T. Shinoda, T. Okuyama, and A. Shimizu. 1983. Comparative studies on the structure of the light chains of human immunoglobulins. IV. Assignment of a sub-subgroup. *J. Biochem. (Tokyo).* 93:421–429.

27. Anderson, M. L. M., M. R. Szajnert, J. C. Kaplan, L. McColl, and B. D. Young. 1984. The isolation of a human Ig Vλ gene from a recombinant library of chromosome 22 and estimation of its copy number. *Nucleic Acids Res.* 12:6647–6661.

28. Guilbert, B., G. Dighiero, and S. Avrameas. 1982. Naturally occurring antibodies against nine common antigens in human sera I. Detection, isolation and characterization. *J. Immunol.* 128:2779–2787.

29. Bona, C. A. 1988. V genes encoding autoantibodies: molecular and phenotypic characteristics. *Annu. Rev. Immunol.* 6:327–358.

30. Schroeder, H. W., Jr., J. L. Hillson, and R. M. Perlmutter. 1987. Early restriction of the human antibody repertoire. *Science (Wash. DC).* 238:791–793.

31. Shlomchik, M. J., A. Marshak-Rothstein, C. B. Wolfowicz, T. L. Rothstein, and M. G. Weigert. 1987. The role of clonal selection and somatic mutation in autoimmunity. *Nature (Lond.).* 328:805–811.

32. Behar, S. M., and M. D. Sharff. 1988. Somatic diversification of the S107 (T15) V_H 11 germ-line gene that encodes the heavy-chain variable region of antibodies to double-stranded DNA in (NZB × NZW) F1 mice. *Proc. Natl. Acad. Sci. USA.* 85:3970–3974.