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Altered cord blood γδ T cell repertoire in Nigeria: possible impacts of environmental factors on neonatal immunity

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Abstract

Infectious diseases during pregnancy can impact the development of fetal immunity, leading to reduced neonatal resistance to infection and decreased responses to pediatric vaccines. *P. falciparum* causes placental infection in low parity pregnant women and is among the pathogens that affect fetal immunity. Recognizing the relationship between malaria and γδ T lymphocytes in adults, we asked whether neonatal γδ T cells would be altered in malaria-endemic regions as a marker for changes in fetal immunity. Our initial studies compared cord blood γδ T cells from deliveries to HIV- mothers in Jos (Nigeria) where malaria is endemic, or in Rome (Italy). We noted substantial differences in the Vγ2 repertoire for cord blood collected in Jos or Rome; differences were consistent with a negative selection mechanism operating on the fetal Vγ2 chain repertoire in neonates from Jos. A specific disruption affected the fraction of γδ T cells that we expect will respond to Bacille Calmette-Guerin (BCG). Fetal γδ T cell depletion might be a mechanism for impaired neonatal immunity and lowered responses to pediatric vaccines.

Keywords

cord blood; gammadelta T lymphocytes; Vγ2 repertoire; Jγ1.2; Nigeria

Introduction

Microbial antigens can cross the placental barrier and prime fetal immunity, often creating immunological memory that persists into early childhood (Malhotra et al., 1999). However, in some cases prenatal priming tolerizes the neonatal immune system and lowers resistance to pathogen exposure in the infant. Placental malaria for example, generates fetal CD4 T-regulatory cells specific for plasmodial antigens and these cells, through IL10 production, suppress protective neonatal T cell responses against Plasmodium (Brustoski et al., 2006) and possibly B cell responses as well (Dent et al., 2006). Children born to mothers with placental

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malaria have increased susceptibility to malaria in early childhood (Le Hesran et al., 1997). Similarly, *in utero* sensitization to helminthes suppresses neonatal responses to helminthes (Hitch et al., 1997; Lammie et al., 1991; Steel et al., 1994) but it also decreases responses to mycobacterium BCG vaccination through a non-specific mechanism (Malhotra et al., 1999). Since we know that γδ T cells comprise an important component of T cell responses to BCG and also respond to plasmodia in adult individuals (Dieli et al., 2001), we decided to explore whether maternal infectious diseases impact neonatal γδ T cells. *In utero* sensitization to pathogens might affect fetal γδ T cell responses either directly, by altering the γδ T cell repertoire, or indirectly by altering neonatal accessory/antigen-presenting cells.

One subset of γδ T lymphocytes, Vγ2Vδ2+ cells are present at low frequency in thymus or cord blood (Casorati et al., 1989; Parker et al., 1990), where a dominant fraction expresses the Vδ1 chain paired mostly with Vγ1 (Morita et al., 1994). Cord blood also contains cells expressing gamma delta chain combinations that are infrequent in adult peripheral blood, such as Vγ2Vδ1 or Vγ1Vδ2 (Morita et al., 1994). Within a few years after birth, the number of Vγ2Vδ2 T cells increases because of strong selection for the Vγ2-Jγ1.2 rearrangement (Parker et al., 1990). It is believed that stimulation by self- or ubiquitous non-self antigens amplifies and maintains the Vγ2-Jγ1.2Vδ2 population, thus creating the biased adult repertoire (Davodeau et al., 1993b; Parker et al., 1990).

Vγ2Vδ2 T cells are stimulated by small non-peptidic compounds collectively called phosphoantigens (Tanaka et al., 1995; Tanaka et al., 1994). Many of these molecules are precursors of isoprenoid biosynthesis (Eberl et al., 2003) such as isopentenyl-pyrophosphate (IPP), and are ubiquitous in eukaryotes. Phosphoantigen recognition is TCR-dependent (Bukowski et al., 1998; Morita et al., 1995) but direct binding to the TCR has not been confirmed with biophysical studies and no antigen presenting molecules are known. Aminobisphosphonates also elicit robust Vγ2Vδ2 lymphocyte responses but act indirectly, by blocking isoprenoid biosynthesis and inducing IPP accumulation (Gober et al., 2003). Responses to phosphoantigens are MHC unrestricted (Morita et al., 1995; Schild et al., 1994) and depend on the Vγ2-Jγ1.2 rearrangement since clones bearing other J segments are less reactive (Davodeau et al., 1993a).

Cord blood (CB) Vγ2Vδ2 T cells are considered to be immature because they have naïve phenotypes and display poor proliferative (Montesano et al., 2001) or cytokine responses (Engelmann et al., 2006) to IPP stimulation. However, CB Vδ2 T cells proliferate robustly after treatment with pamidronate or live mycobacteria (BCG), thus they are not inherently unresponsive (Cairo et al., 2008). As a first step toward understanding the mechanisms controlling Vγ2Vδ2 T cells in CB, we compared the repertoire of Vγ2, Vδ2 and Vδ1 chains for CB samples collected in Jos, Nigeria and in Rome, Italy.

Malaria is endemic in Nigeria and prevalence among pregnant women in Jos was reported to be approximately 40% (Egwunyenga et al., 2001; Egwunyenga et al., 1997). In other areas of Nigeria, between 70 and 80% of pregnant women were positive for *Plasmodium* even in the absence of symptoms (Onyenekwe et al., 2002). Such a prevalence can be explained by a high incidence and/or long duration of infection. Treatment or previous exposures may decrease the duration of infection and re-infection in not easily distinguished from recrudescence in the absence of molecular typing for the parasites. In a setting that mimicked transmission to a naïve host in an endemic region, untreated *P. falciparum* infection lasted on average about 200 days (Macdonald, 1950; Sama et al., 2006) but some studies found evidence for longer (Sama et al., 2004) or shorter (Sama et al., 2006) parasite persistence in humans that were exposed repeatedly. If we consider a duration of approximately 200 days and a prevalence of 40%, we can appreciate that most women will suffer from *Plasmodium* infection at least once during

pregnancy. Although we did not measure specific parasite burden in maternal blood, it is likely that our samples from Jos reflect this common situation.

Our goal in this study was to collect CB from a random sample of deliveries in a population at high risk for malaria, then look for similarities and differences between neonatal γδ T cells in high (central African) or low risk (European) populations. Our results are consistent with the idea that environmental factors alter fetal Vγ2Vδ2 T cells; likely mediators include malaria and other endemic tropical diseases.

Materials and Methods

Lymphocyte sample collection

Umbilical cord blood samples were obtained by venipuncture of the umbilical vein immediately after uncomplicated full-term vaginal deliveries. Samples were collected at San Pietro-Fatebenefratelli Hospital, in Rome, Italy, and Plateau State Hospital, in Jos, Nigeria. In both sites women donating CB were HIV-. Women enrolled in Rome did not have symptoms of infectious disease during pregnancy; infectious disease history was not known for women enrolled in Jos. HIV prevalence in Jos is approximately 10% among pregnant women (Sagay et al., 2006). HIV serostatus for Nigerian women was confirmed during labor using two different rapid tests, Determine (Abbott) and Stat-Pak (Chembio). In case of discordance, the Genie-II (Sanofi) test was used as a tie-breaker.

Informed consent was obtained from all women donating cord blood. Cord blood mononuclear cells (CBMC) were isolated by centrifugation over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient according to the manufacturer's instructions.

RNA extraction, RT- PCR, PCR

Total RNA was extracted from $1–10\times10^6$ cells using the RNeasy mini Kit (Qiagen, Valencia, CA), as described by the manufacturer. One μg of total RNA was then converted into cDNA using the reverse transcription system kit (Promega, Madison, WI), as described previously (Cairo et al., 2007). Polymerase chain reactions were performed as described (Cairo et al., 2007) using the following primers: for the Vγ2 (Vγ9 according to the IMGT nomenclature) chain, oligo Vγ2 (5' ATC AAC GCT GGC AGT CC 3') and oligo Cγ-1 (5' GTT GCT CTT CTT TTC TTG CC 3'); for the Vδ2 chain, oligo Vδ2 (5' GCC ATT GAG TTG GTG CCT GAA C 3') and oligo Cδ-1 (5' TGG CAG TCA AGA GAA AAT TG 3'); for the Vδ1 chain oligo Vδ1 (5' TTA ACC ATT TCA GCC TTA CAG C 3') and oligo Cδ-1 (5' TGG CAG TCA AGA GAA AAT TG 3'). PCR products were separated on 1% agarose/Tris-acetate-EDTA buffer (TAE) gels containing 0.5μg/ml ethidium bromide.

Run-off reaction

Primer extension reactions were performed as described (Evans et al., 2001). Each reaction contained 2μl of PCR product, 3 mM $MgCl_2$, 0,2 mM dNTP, 0.1 μM of 6-carboxyfluorescein (6-FAM) labeled primer (Cγ-6: 5'-6-FAM–AAT AGT GGG CTT GGG GGA AAC-3'; Jγ1.2: 5'-6-FAM–; Cδ: 5'-6-FAM–ACG GAT GGT TTG GTA TGA GG-3'), approximately 0.2 units of Taq DNA polymerase (Promega, Madison, WI), 10 mM Tris pH8.8, and 50 mM KCl. 4μl of run-offs products were diluted with 6μl deionized formamide (Applied Biosystems, Foster City, CA), and 0.7μl of GeneScan-500 ROX size standard were added to each sample. After a denaturation step (5 min at 95°C followed by immediate quenching on ice) products were loaded on a 3130 genetic analyzer (Applied Biosystems, Foster City, CA) and run on a performance-optimized polymer (POP-7). Molecular size and relative frequency of extension products were determined using GeneMappaer software (Applied Biosystems, Foster City, CA). In order to standardize the data irrespective of the run-off primer position, CDR3 length

variation was expressed in terms of the total Vγ2, Vδ2 and Vδ1 coding region lengths. Run-off product lengths were corrected by adding the length of the known mRNA coding region outside the run-off primer-binding site. For Vδ2 and Vδ1 chains, the sequences used as reference for calculations are those listed in the IMGT website (accession number: **M22198** for Vδ1; **X15207** for Vδ2; **M22148**, **M22149** and **M22150** for Cδ), plus the sequence for the human alpha/delta locus (accession **NG 001332**). For the Vγ2 chain, according to our convention (Evans et al., 2001) the peak at 993 nucleotides corresponds to a product 444 nucleotide long.

Cloning and sequencing of Vγ2 chains

PCR products for the Vγ2 chain were purified by gel extraction, using QIAquick gel extraction kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. Purified products were denatured (1 minute at 94°C), then incubated for 30 minutes at 72°C with 2mM $MgCl_2$, 0.2mM dATPs, and 2.5 units of Amplitaq gold, then ligated into a pCR2.1 vector. Ligated vector was transfected into TOP 10F' competent cells (TA cloning kit, Invitrogen, Carlsbad, CA), and bacterial colonies representing a library of Vγ2 chain sequences were grown overnight on agar plates containing 50μg/ml ampicillin, 500 μM IPTG and 80μg/ml X-Gal. Colonies containing recombinant plasmids were cultured overnight in LB media and plasmid DNA were purified using the REAL Minipreps DNA purification kit (Qiagen, Valencia, CA). Sequencing reactions were done with a Big Dye v3.1 fluorescent sequencing kit (Applied Biosystems, Foster City, CA), with both M13F and M13R oligonucleotide primers for each sample. Sequences were loaded on an automated sequencer ABI3700 and analyzed using Sequencher and MacClade softwares. We were unable to unambiguously identify C1 and C2 chains based on the C sequence that we determined, hence the Jγ1.3 and Jγ2.3 segments (J1 and J2, respectively, according to the IMGT nomenclature) were not distinguished and were pooled together as Jγ1.3/2.3

Statistical analysis

Differences among groups were evaluated using two-tailed Student's t test. $p < 0.05$ was considered significant.

Results

γδ T cells for CB collected in Rome or Jos differed in Vγ2 chain length distributions

As a test for differences in the neonatal γδ TCR repertoire between CB samples collected in Rome (CB-R) or Jos (CB-J), we compared spectratype data for 35 Italian and 17 Nigerian CB specimens. The Vδ1 chain length distributions were similar for both groups, with mode at 882 nucleotides (nt) (Fig 1A). The Vδ2 chain lengths were also similar (modal value = 870 nt) although some longer chains were more frequent in CB-J (Fig 1B). The Vγ2 (Vγ9 according to the IMGT nomenclature) chain length distributions for both CB groups were bimodal, the first peak being 984 and the second 993 nt (Fig 2A). CB-R had a higher frequency of chains between 990 and 996 nt compared to CB-J ($p=0.01$); this was of interest because most Vγ2 chains in the 990–996 length range from adults include the Jγ1.2 (JP according to the IMGT nomenclature) segment (Evans et al., 2001). About 90% of the Jγ1.2+ chains fell in the 990–996 length range for both CB groups (Fig 2B).

The Vγ2 repertoire for CB samples collected in Rome had higher Jγ1.2 frequency and lower population complexity

Based on spectratyping results, we chose 6 representative CB-R and 5 CB-J specimens, then created Vγ2 cDNA libraries from mRNA. For each sample, we sequenced between 200 and 300 Vγ2 cDNA clones in order to have sequences for at least 100 productively rearranged

chains from each specimen. For all CB samples, Vγ2 chain length distributions determined by cDNA sequencing agreed with distributions measured by spectratyping (data not shown). In particular, 92.2%±3.1% (for CB-R) and 84.2%±12.5% (for CB-J) of the Vγ2-Jγ1.2 chains fell into the 990–996 length range. Among all Vγ2 chains between 990 and 996 nt long, 79.3% ±9.8% were Jγ1.2+ for CB-R, but only 45.6%±11.8% were Jγ1.2+ for CB-J ($p<0.001$).

Table 1 shows an overview of sequencing results for productively rearranged Vγ2 chains from both CB groups. The frequency of Vγ2-Jγ1.2 nucleotide sequences (or nucleotypes) was significantly higher for CB collected in Rome (41.5%±15.3% vs. 13.9%±8.5%, $p=0.01$). The Vγ2 repertoire complexity (defined as: number of different Vγ2 nucleotypes ÷ number of total Vγ2 sequence entries) was higher for CB-J. Because of the way complexity is defined, a repertoire dominated by nucleotypes that are present multiple times (repeated) will have lower complexity, while a repertoire dominated by unique nucleotypes (that occur only once in the pool) will have higher complexity. The two CB groups showed similar complexities for the subset of Vγ2 chains that include Jγ1.3 or 2.3 segments (Vγ2-Jγ1.3/2.3 complexity = number of different Jγ1.3/2.3 nucleotypes ÷ number of total Jγ1.3/2.3 sequence entries). When we examined the subset with Vγ2-Jγ1.2 chains, complexity was significantly lower for CB-R (0.55 ±0.1 for CB-R vs 0.8±0.17 for CB-J, $p=0.015$). Lower complexity often indicates a repertoire bias caused either by preferential recombination or positive selection mechanisms that allow individual V-J rearrangement to accumulate.

Particular Jγ1.2 nucleotypes were abundant (Supplementary Material S1 and S2); the most common was a canonical sequence (where canonical refers to any sequence that lacks N or P nucleotides and is entirely encoded by the germ-line) that was detected previously in fetal liver (McVay and Carding, 1996) and in CB (Delfau et al., 1992). This nucleotype was the only one present in all CB samples and its frequency within the Vγ2-Jγ1.2 pool ranged from 7.8% to 45.5% (Table 2). The proportion of this canonical nucleotype within the entire Vγ2 pool was significantly lower for CB-J samples, although its proportion within the Jγ1.2 subset was similar in both groups (Table 2). The high frequency of a canonical nucleotype in CB-R specimens is consistent with preferential production and/or positive selection of these cell clones in CB-R specimens. Assuming that the same recombination/selection mechanisms would also occur in the CB-J samples, differences for the Jγ1.2 subsets are likely to result from negative selection in the CB-J specimens.

The number and proportion of public Vγ2-Jγ1.2 clonotypes is higher for CB collected in Rome

For each specimen, we analyzed the deduced aminoacid sequences (clonotypes). In the Jγ1.2 and Jγ1.3/2.3 subsets we observed clonotypes that were present in more than one CB specimen (public clonotypes). The number of public Jγ1.2 clonotypes was higher for CB-R than for CB-J (23 versus 5) and the fraction of Jγ1.2 chains encoding public clonotypes was also higher (on average 68.5% for CB-R and 38.3% for CB-J, $p<0.001$) (Table 3). CB-R had a similar number of public Jγ1.3/2.3 clonotypes compared to CB-J (21 versus 26), and the fraction of Jγ1.3/2.3 chains encoding public clonotypes was the same for both (Table 4). Several public Jγ1.2 clonotypes were abundant, while public Jγ1.3/2.3 clonotypes were found only 3 times or less in any sample.

We also noticed that some clonotypes (mostly public) were encoded by more than one nucleotype; these are examples of sequence convergence. For CB-R, convergence occurred mainly among Jγ1.2 chains; for CB-J it occurred mostly among Jγ1.3/2.3 clonotypes (Supplementary Material, S3); this may be due to an overall lower proportion of Jγ1.2 clonotypes in CB-J. These data suggest that positive selection was operating mainly on the Vγ2-Jγ1.2 subset for CB-R specimens. Assuming that the same positive selection was operating in CB-J, the differences between Vγ2Jγ1.2 subsets in CB-R and CB-J are consistent with

negative selection that limited or reversed the impact of an underlying positive selection on Vγ2-Jγ1.2 chains in CB-J.

Discussion

Using Vγ2Vδ2 T lymphocytes as a model population, we sought to understand how the fetal immune system is affected by maternal infectious diseases. In this work, we used detailed molecular characterization of CB γδ T cells to measure similarities and differences between specimens collected in Jos, Nigeria, or Rome, Italy. This is our initial step toward understanding environmental factors that affect fetal immunity.

In both CB groups, the Jγ1.3/2.3 subset showed characteristics of an unselected, naïve repertoire: high complexity, absence of prominent clones, and a low fraction of nucleotypes encoding public clonotypes. Importantly, the Jγ1.3/2.3 subset was very similar between the two groups. These results argue strongly against inherent differences in the Vγ2-Jγ1.3/2.3 cell populations and support the view that other differences are likely the result of selection mechanisms. We cannot exclude the impact of genetic factors on the observed repertoire differences. At present, we are studying the cord blood repertoire from African Americans to search for possible genetic confounders. Comparative studies to date of adult Vγ2 repertoire in European, North American and Chinese populations has not revealed any relationship between ethnicity and γδ T cells.

CB-R specimens (cord blood from Rome) had higher Vγ2-Jγ1.2+ chain frequencies and lower complexity compared to CB-J. These differences were due mostly to the low frequency of the dominant Jγ1.2 canonical nucleotype in CB-J specimens. CB-R specimens were characterized by higher proportions of Vγ2-Jγ1.2+ nucleotypes that encode public clonotypes, including the dominant canonical sequence. Though CB γδ T cell populations are still immature compared to the adult repertoire, CB-R specimens showed a pattern consistent with positive selection operating on the Vγ2 chain repertoire and mainly selecting for Vγ2-Jγ1.2+ cells.

The dominant canonical Vγ2-Jγ1.2 sequence, first produced during early ontogeny and detectable in prethymic fetal liver (McVay and Carding, 1996), is common to many human cord or adult blood samples (Davodeau et al., 1993b; Delfau et al., 1992; McVay and Carding, 1999) and its homolog is also common in macaques (Cairo et al., 2007; MacDougall et al., 2001; Rakasz et al., 2000). This sequence, like other canonical nucleotypes, could be generated by homology-mediated recombination (Asarnow et al., 1993; Itohara et al., 1993; Zhang et al., 1995). Such a mechanism may explain why this sequence is common even across species. Few of our Jγ1.2 and 1.3/2.3 canonical nucleotypes are compatible with a directed recombination mechanism. When present, sequences that might result from directed recombination were at frequencies lower than the dominant Jγ1.2 canonical nucleotype. Recombination mechanisms alone do not dictate the frequency of canonical sequences. It is likely that preferential survival allows expansion of clones expressing canonical Vγ2 chains and thus, shapes the fetal Vγ2 repertoire.

Whether the dominant canonical sequence arises by preferential recombination, positive selection or both, we expect processes shaping the Vγ2 repertoire to be similar for fetuses in Jos and Rome. However, a surfeit of stimulation occurring in the presence of high antigen concentration could result in depletion of the fetal Vγ2Jγ1.2+ subset. This mechanism would give the appearance of induced tolerance, because reactive Vγ2+ clones would be lost due to activation-induced cell death. Thus, environmental factors in Jos might be driving specific depletion of Vγ2Jγ1.2+ cells during fetal development and the differences between Jos and Rome might be explained by the type and intensity of signaling among fetal lymphocytes. The

absence of effects on Vγ2-Jγ1.3/2.3 cells, which do not react to phosphoantigens, is consistent with an antigen-specific tolerance mechanism.

We focused on malaria as a possible source of Vg2Vd2 T cell stimulation, for three main reasons. First, malaria prevalence is high in Jos (Egwunyenga et al., 2001; Egwunyenga et al., 1997). Second, malaria has documented effects on adult γδ T cells, including expansion of peripheral blood subsets for endemic infections (Hviid et al., 2001; Worku et al., 1997) and specific depletion of Vγ2Jγ1.2 cells in naïve individuals (traveler's malaria) (Martini et al., 2003); fetuses are immunologically naïve and plasmodia exposure might have an impact similar to infection of naïve adults. Third, *P. falciparum* produces small stimulatory phosphoantigens (Behr et al., 1996) (approximately 200 dalton) that might easily cross the placental barrier and trigger fetal responses.

Tolerance is the functional manifestation of negative selection that occurs when antigen-stimulated T cell clones are deleted. It is likely that fetal exposure to pathogens and their phosphoantigens will activate Vγ2-Jγ1.2+ cells including those with canonical or public clonotypes, and this potentially prolonged stimulation may promote tolerance, that we observe here as repertoire disruption. The importance of these mechanisms may be most apparent during the first year of life. A Vγ2-Jγ1.2 repertoire depletion might remove a T cell subset critical for the response to pediatric vaccination against tuberculosis. Documented poor performance of the tuberculosis vaccine (BCG) in equatorial vs. non-equatorial regions (Colditz et al., 1994) suggests some effects of environment on host response; tolerance in the γδ T cell population may be one mechanism for affecting newborn immunity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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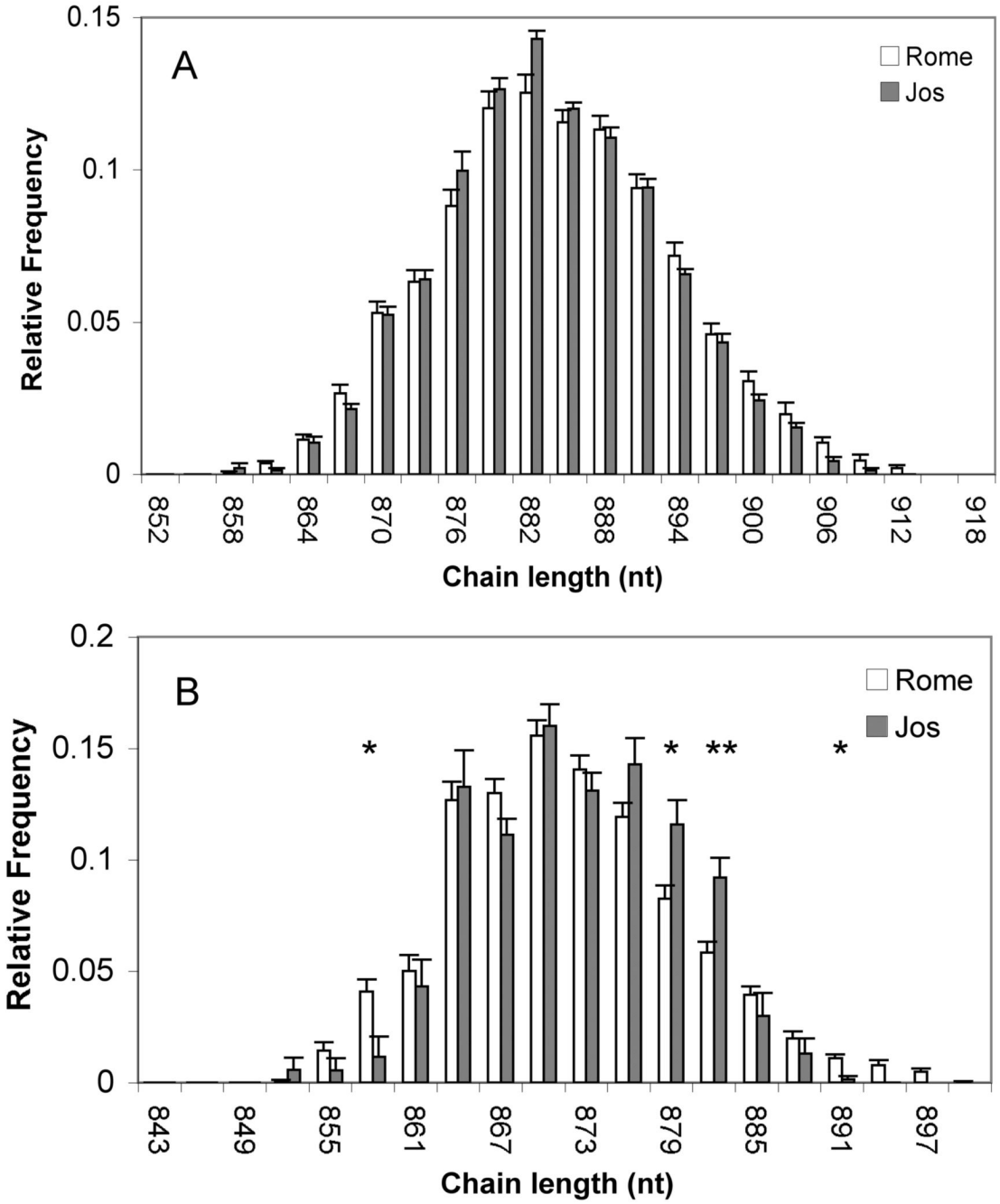

Figure 1. Vδ1 and Vδ2 chain length distributions are similar for CB-R and CB-J

Vδ1 (A) and Vδ2 (B) chain length distributions were analyzed by spectratyping for 34 CB collected in Rome and 17 CB collected in Jos. Panels show, for each chain length, mean frequency + SE. *=$p<0.01$; **=$p<0.001$

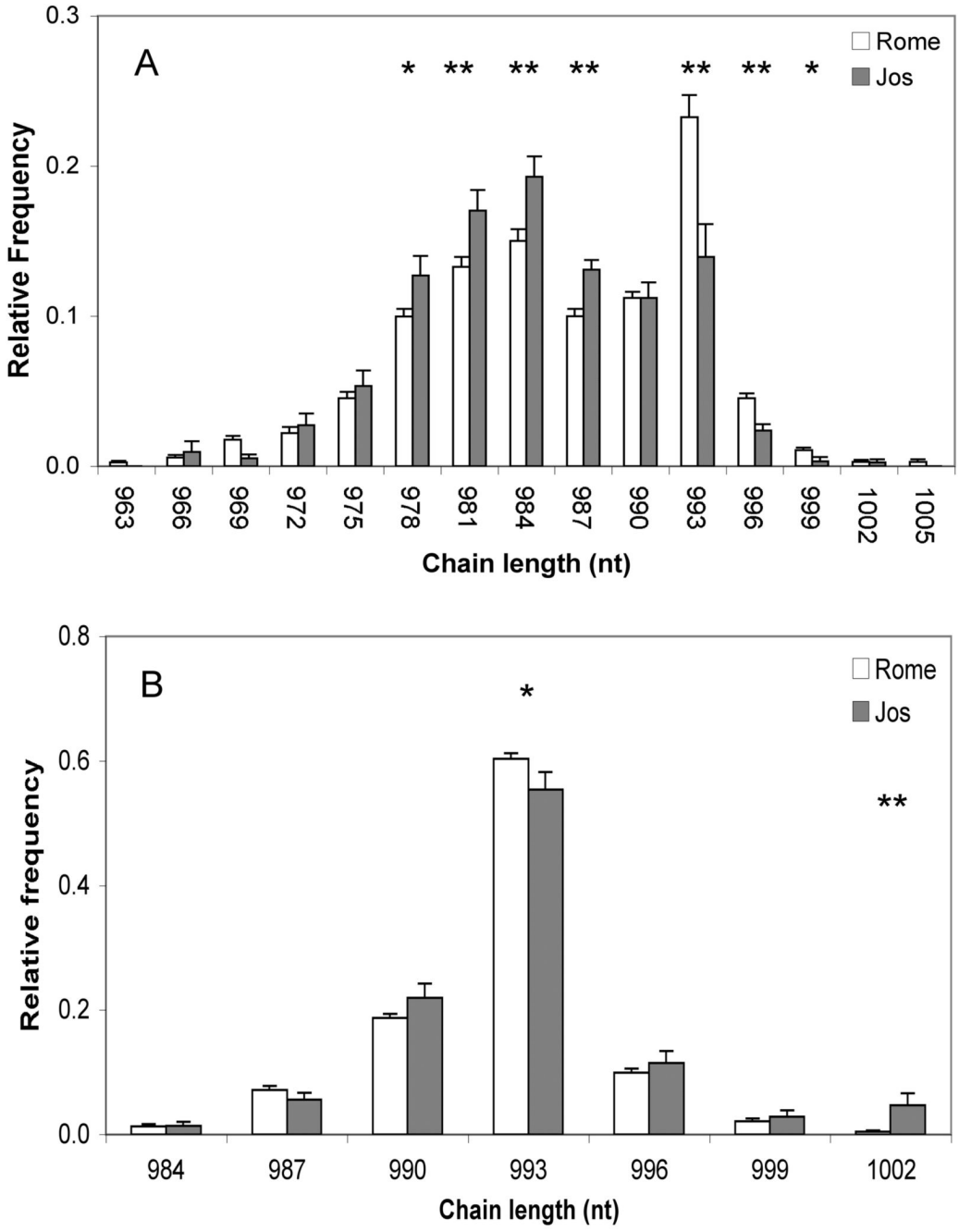

Figure 2. The frequency of Vγ2 chains in the 900–996 nt range is higher for CB-R

Vγ2 chain length distribution (A) was analyzed by spectratyping for 34 CB collected in Rome and 17 CB collected in Jos. The distribution of Jγ1.2+ chains (B) was also examined by using a Jγ1.2 specific primer. Panels show, for each chain length, mean frequency + SE. *=p<0.05; **=p<0.01

Table 1

CB samples collected in Nigeria have a lower Jγ1.2 frequency and higher population complexity

		CCB22	CCB23	CCB29	CCB35	CCB38	CCB42	Average
Rome	Jγ1.2	51 (36.7%)	33 (29.2%)	93 (66.4%)	67 (45.3%)	17 (18.9%)	76 (52.4%)	41.5%
	Jγ1.3/2.3	81 (58.3%)	72 (63.7%)	43 (30.7%)	78 (52.7%)	63 (70%)	57 (39.3%)	52.5%
	Jγ1.1	6 (4.3%)	8 (7.1%)	4 (2.9%)	3 (2%)	5 (5.5%)	8 (5.5%)	4.6%
	Jγ2.1	1 (0.7%)	nd	nd	nd	5 (5.5%)	4 (2.8%)	1.5%
	Total #	139	113	140	148	90	145	129
	Vγ2 Complexity	0.75	0.72	0.68	0.79	0.73	0.71	0.73
	Jγ1.2 Complexity	0.65	0.36	0.56	0.63	0.53	0.55	0.55
	Jγ1.3/2.3 Complexity	0.79	0.88	0.91	0.92	0.81	0.88	0.87

		NCB4	NCB5	NCB7	NCB18	NCB19	Average
Jos	Jγ1.2	12 (9.5%)	8 (7%)	11 (8%)	25 (16.1%)	43 (28.9%)	13.9%
	Jγ1.3/2.3	108 (85.7%)	104 (90.4%)	120 (87%)	119 (76.8%)	91 (61%)	80.2%
	Jγ1.1	3 (2.4%)	2 (1.7%)	2 (1.5%)	6 (3.9%)	7 (4.7%)	2.8%
	Jγ2.1	3 (2.4%)	1 (0.9%)	4 (2.9%)	4 (2.6%)	8 (5.4%)	2.8%
	Total #	126	115	138	155	149	137
	Vγ2 Complexity	0.92	0.8	0.92	0.75	0.79	0.84
	Jγ1.2 Complexity	0.92	1	0.82	0.64	0.61	0.80
	Jγ1.3/2.3 Complexity	0.92	0.78	0.93	0.78	0.88	0.86

The table reports, for every CB specimen, the number (percentage) of chains bearing each J segment, the total number of productively rearranged Vγ2 chains and population complexity for the Vγ2 pool as well as for the two major subsets, the Jγ1.2 and the Jγ1.3/2.3. Complexity is calculated as: number of different nucleotyes in a sample set ÷ total number of nucleotypes in the sample set

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Table 2

The canonical nucleotype (GGGAGGTG CAAGAG) frequency in the Vγ2 pool is lower for CB-J, but within the Jγ1.2 subset it is similar for CB-R and CB-J

		% within Jγ1.2	% within Vγ2
Rome	CCB22	7.8	3.6
	CCB23	45.5	13.3
	CCB29	33.3	22.1
	CCB35	29.9	13.5
	CCB38	41.2	7.7
	CCB42	32.8	17.2
	Average	**31.8**	**12.9**
Jos	NCB4	8.3	0.8
	NCB5	12.5	0.9
	NCB7	27.3	2.2
	NCB18	36	5.8
	NCB19	39.5	11.4
	Average	**24.7**	**4.2**

Table 3

The fraction of public Jγ1.2 clonotypes is lower for CB-J than for CB-R

A

Vγ2	N	Jγ1.2	CCB22	CCB23	CCB29	CCB35	CCB38	CCB42
CALWE	----AL--QELGKKIKVFGPGTKLIIT				1			1
CALWE	----D---QELGKKIKVFGPGTKLIIT			1	1			
CALWE	----E----ELGKKIKVFGPGTKLIIT		1		1			
CALWE	---------ELGKKIKVFGPGTKLIIT		3				1	2
CALWE	----G---QELGKKIKVFGPGTKLIIT			1		1		
CALWE	----I---QELGKKIKVFGPGTKLIIT		1			1		2
CALWE	----K----ELGKKIKVFGPGTKLIIT		1		1			
CALWE	----P---QELGKKIKVFGPGTKLIIT		2		1			3
CALWE	--------QELGKKIKVFGPGTKLIIT		4	5	3	1		
CALWE	----R---QELGKKIKVFGPGTKLIIT				2			2
CALWE	----S---QELGKKIKVFGPGTKLIIT				1			1
CALWEV	---E----ELGKKIKVFGPGTKLIIT				1	2		1
CALWEV	--------ELGKKIKVFGPGTKLIIT			3	2	3		3
CALWEV	---G----ELGKKIKVFGPGTKLIIT		3					1
CALWEV	---H----ELGKKIKVFGPGTKLIIT					2	1	1
CALWEV	---K----ELGKKIKVFGPGTKLIIT				3	1	1	
CALWEV	---L----ELGKKIKVFGPGTKLIIT				1	2		1
CALWEV	-------QELGKKIKVFGPGTKLIIT		7	15	33	22	10	27
CALWEV	---Q-----LGKKIKVFGPGTKLIIT				3	1		1
CALWEV	---R----ELGKKIKVFGPGTKLIIT		5		4	1		7
CALWEV	---RG---ELGKKIKVFGPGTKLIIT		1					1
CALWEV	---R---QELGKKIKVFGPGTKLIIT				1			1
CALWEV	---RS---ELGKKIKVFGPGTKLIIT				1		2	
Total # of public Jγ1.2			28	25	60	37	15	55
Total # of Jγ1.2			51	33	93	67	17	76
Fraction of public Jγ1.2 (%)			54.9	75.8	64.5	55.2	88.2	72.4

B

Vγ2	N	Jγ1.2	NCB4	NCB5	NCB7	NCB18	NCB19
CALWE	----A---QELGKKIKVFGPGTKLIIT			1			1
CALWE	----D---QELGKKIKVFGPGTKLIIT		1		1		
CALWE	----------LGKKIKVFGPGTKLIIT		1				1
CALWE	--------QELGKKIKVFGPGTKLIIT			1			3
CALWEV	-------QELGKKIKVFGPGTKLIIT		1	1	3	10	17
Total # of public Jγ1.2			3	3	4	10	22
Total # of Jγ1.2			12	8	11	25	43
Fraction of public Jγ1.2 (%)			25.0	37.5	36.4	40.0	51.2

All public Vγ2-Jγ1.2 clonotypes are listed for both CB-R (A) and CB-J (B). The number of occurrences is reported for every clonotype and the fraction of Jγ1.2 sequences coding public clontypes is calculated as: total number of Jγ1.2 sequences ÷ total number of Jγ1.2 sequences coding public clonotypes.

Table 4

The fraction of public Jγ1.3/2.3 clonotypes is similar for CB-J than for CB-R.

A

Vγ2	N	Jγ1.3/2.3	CCB22	CCB23	CCB29	CCB35	CCB38	CCB42
CALWE---AP---NYYKKLFGSGTTLVVT						1		1
CALWE---D-------KKLFGSGTTLVVT			1			1		
CALWE---D-----YYKKLFGSGTTLVVT					1	2		1
CALWE---G-------KKLFGSGTTLVVT						1		1
CALWE---LS---NYYKKLFGSGTTLVVT			1	1		1		
CALWE---M----NYYKKLFGSGTTLVVT			1				1	
CALWEV--E--------KLFGSGTTLVVT						1		2
CALWEV--F-----YYKKLFGSGTTLVVT			1			1		
CALWEV--GG----YYKKLFGSGTTLVVT				1			3	
CALWEV--G------YKKLFGSGTTLVVT			1	2			1	
CALWEV--H------YKKLFGRGTTLVVT			2		1			
CALWEV----------KKLFGSGTTLVVT					1		3	
CALWEV--L-----YYKKLFGSGTTLVVT			2	1			3	
CALWEV-------NYYKKLFGSGTTLVVT			1	1	1	2		2
CALWEV--QG-------KLFGSGTTLVVT				1			1	
CALWEV--RG-----YKKLFGSGTTLVVT			1					1
CALWEV--R-----YYKKLFGSGTTLVVT				1	2			1
CALWEV--T-----YYKKLFGSGTTLVVT				1		1		
CALWEV--V------YKKLFGSGTTLVVT			1			1		
CALWEV--------YYKKLFGSGTTLVVT				2	1		1	1
CALWE---------YYKKLFGSGTTLVVT					1		2	
Total # of public Jγ1.3/2.3			12	11	8	12	10	10
Total # of Jγ1.3/2.3			81	72	43	78	57	57
Fraction of public Jγ1.3/2.3 (%)			14.8	15.3	18.6	15.4	23.8	17.5

B

Vγ2	N	Jγ1.3/2.3	NCB4	NCB5	NCB7	NCB18	NCB19
CALWE---A----NYYKKLFGSGTTLVVT			1		1	1	
CALWE---A-----YYKKLFGSGTTLVVT				1			2
CALWE---D-----YYKKLFGSGTTLVVT				1	2	1	
CALWE---G----NYYKKLFGSGTTLVVT					1		1
CALWE---G------YKKLFGSGTTLVVT			1		1		
CALWE---G-----YYKKLFGSGTTLVVT			1		1		
CALWE---LD----YYKKLFGSGTTLVVT				1	1		
CALWE---L----NYYKKLFGSGTTLVVT					1	1	
CALWE---LP----YYKKLFGSGTTLVVT					1	1	
CALWE--------NYYKKLFGSGTTLVVT			2	1	1		
CALWE---PR---NYYKKLFGSGTTLVVT					1		1
CALWE---S----NYYKKLFGSGTTLVVT					1	1	
CALWE---S-----YYKKLFGSGTTLVVT			1		1		1
CALWEV--G------YKKLFGSGTTLVVT				1	1		
CALWEV--H-----YYKKLFGSGTTLVVT			1	1		1	
CALWEV--IN------KKLFGSGTTLVVT			1	1			
CALWEV---------KKLFGSGTTLVVT				2		1	

B

Vγ2	N	Jγ1.3/2.3	NCB4	NCB5	NCB7	NCB18	NCB19
CALWEV--KY------KKLFGSGTTLVVT			1		1		
CALWEV--L------YKKLFGSGTTLVVT			1		1		
CALWEV--L-----YYKKLFGSGTTLVVT			3		1		
CALWEV-------NYYKKLFGSGTTLVVT			2	1			
CALWEV--P-----YYKKLFGSGTTLVVT			3	3			1
CALWEV--QG----YYKKLFGSGTTLVIT					1	1	
CALWEV--R-------KKLFGSGTTLVVT				2	1		2
CALWEV--R------YKKLFGSGTTLVVT			2		2	2	1
CALWEV--------YYKKLFGSGTTLVVT					2		1
Total # of public Jγ1.3/2.3			20	15	23	10	10
Total # of Jγ1.3/2.3			108	104	120	119	91
Fraction of public Jγ1.3/2.3 (%)			18.5	14.4	19.2	8.4	11.0

All public Vγ2-Jγ1.3/2.3 clonotypes are listed for both CB-R (A) and CB-J (B). The number of occurrences is reported for every clonotype and the fraction of Jγ1.3/2.3 sequences coding public clontypes is calculated as: total number of Jγ1.3/2.3 sequences ÷ total number of Jγ1.2/2.3 sequences coding public clonotypes.