neopeptide in the thymus provides a new approach for exploring the role of peptide in the positive selection of T cells. This strategy inverts the traditional one of starting with a T cell displaying a particular TCR and then attempting to define the requirements for its selection; rather, it begins with expression of a new peptide and permits one to study the T cells naturally selected on it. Our data show that the peptide sequence influences the sequence of the TCRαβ chains, significant and systematic variations resulting from single-residue changes at putative TCR contact points. The relation between selecting peptide and selected TCR shows significant, but not complete, two-way degeneracy, analogous to what is seen with the responses of mature T cells. Taken together, these results point to a way to steric hindrance of the TCR (19).

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5. K. A. Hogquist et al., Cell 76, 17 (1994).
14. T. Miyazaki et al., ibid., p. 531.
18. S. Vivile et al., ibid., 72, 635 (1993). It was observed that mice from a backcross of the lim mutation onto the B10.BR background for three to five generations. Heterozygote controls were littermates or parallel crosses to B10.BR.
26. For the lymph node proliferation assays, 100 ng of peptide was injected with complete Freund's adjuvant into one footpad. Ten days later, draining popliteal lymph nodes were isolated and round-bottom 96-well plates (3 x 10^5 per well) with or without peptide for 72 hours. The cells were pulsed with 1 μCi of [3H]thymidine for the last 18 hours of culture. Peptides used include MCC(88–103) (ANERADLALAYLKQ), and its variants at position 99 or 102, HoB(67–76) (VITAFNGEQKL), LI(12–26) (LEADRRLAKEYRKK), and RNaseA(90–105) (CIKVLASTKHKK). All were synthesized by FMOC (fluorenylmethoxycarbonyl) chemistry and purified by reverse-phase high-performance liquid chromatography.
32. T. Ploegh et al. (1996) present a provocative alternative (20). Briefly, the chimeric Ii MHC class II allele is expressed on the surface of myeloid cells, and the complex is pulsed with a single peptide. After 24 hours, the supernatants were collected and tested for interleukin–2 (IL–2) by proliferation (assayed as [3H]thymidine incorporation) of the IL–2–dependent CTL clone cell line.
33. A detailed description of the adenovirus vectors will be published elsewhere (16). Briefly, the chimeric Ii cDNA fragments were constructed by polymerase chain reaction (PCR) mutagenesis followed by ligation and insertion into the Eco RI–Eco RI sites of PV41. The resulting plasmids were linearized, then transcribed together with the right-hand OA fragment of d324 into 293 cells (which complement the E1A deficiency; American Type Culture Collection CRL1573). Viral plaques stemming from recombination between plasmid and truncated adenoviral sequences were selected, screened for the presence of the li cDNA in the correct conformation, and plaque-purified twice. Large viral stocks were prepared in liquid cultures of 293 cells, purified, and concentrated on CsCl gradients. Purified virus was dialyzed against 0.5 M NaCl, 20 mM tris-HCl (pH 7.6), and stored at -80°C, filtered by limiting dilution (16), and verified. Before injection, virus was diluted three times in RPMI medium. Ten microliters of this dilution were injected into each thymic lobe of anesthetized mice with a Hamilton syringe (21). The titer of all concentrated viral stocks ranged from 0.3 x 10^12 to 1.0 x 10^12 IU/ml.
34. Draining lymph node cells from immunized mice were restimulated in vitro with 0.3 or 1.0 μM MCC(88–103) peptide for 2 days and expanded with IL–2 (50 U/ml) for another day. Blasts were fusomed with α-BW5147 cells (1/2 ratio) and hybrids selected in hypoxanthine, aminopterin, thymidine (HAT) medium. Hybridomas were tested for reactivity to MCC peptide presented by B10.BR splenocytes (32) and were recloned by limiting dilution.
35. For the antagonist assays, the B cell lymphoma line OH27 was treated with mitomycin C (26 μg/ml) for 30 min, pulsed with sub saturating doses of MCC(88–103) peptide for 3 hours at 37°C, and washed three times. These stimulators (3 x 10^6 per well) were cultured with the T cell hybridomas or clones (3 x 10^6 per well) in the presence of various doses of putative antagonist peptides. Hybridoma stimulation was measured as IL–2 production (32). Direct proliferation was measured for the T cell clone upon pulsing with 1 μCi of [3H]thymidine for 18 hours after 48-hour incubation.
38. We thank M. Perricaudet for early help on this project. P. Allen for peptides; P. Marchal for much of the sequencing; M. Gilbert and C. Ebel for cells; F. Fischer, W. Magniant, and the staff of the Centre de Developement des Techniques Avancées-CNRS for maintaining the mice; and P. Gerber for assistance. Supported by institutional funds from the INSERM, the CNRS, the Centre Hospitalier Universitaire Regional, Bristol Myers-Squibb, and by grants to D. M. and C.B. from the Association pour la Recherche sur le Cancer (ARC), and the Human Frontier Science Program. N.N. and R.R. were supported by fellowships from the ARC, CNRS, and Ligue Nationale contre le Cancer (LNCC), and the LNCC and Canadian Medical Research Council, respectively.
39. The data from this study were published in Science on May 10, 2017.
(1); however, the problem is particularly critical in figure 4, which provides the primary data to suggest a relation between the growth of the brain and the detachment of the ossicles. In this figure, Rowe superimposes his data on the growth of the ectotympanic and dentary bones and the date of the detachment of the auditory ossicles in Monodelphis on data on brain growth in Diphyurus presented by Ulinski (5). He does not correct for the differing rates of development; instead, the two data sets are combined. This is equivalent to taking one set of measurements on a domestic cat and another on a tiger and, without correction for size or rates of development, summarizing the “felid” pattern. The auditory ossicles do not detach from Meckel’s cartilage at day 21 in Diphyurus because at this time there is no jaw condyle nor is there ossification of any ossicle (4). Further, all evidence suggests that at 20 days after birth the brain is far more advanced in Monodelphis than in a 20-day Diphyurus pouch young (6). If Rowe is to argue a relation between the timing of events in development, he must either compare data derived from a single species or, at the least, correct for the differing rates of development in two very different species.

Kathleen K. Smith
Alexander F.H. van Nievelt

Department of Biological Anthropology and Anatomy,
Duke University,
Durham, NC 27710, USA

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Response: Do Diphyurus and Monodelphis really have differing rates of growth? In answering this question, care must be taken to distinguish between rates of growth and rates of maturation because the two are broadly correlated but are not strictly coupled throughout ontogeny (1). Diphyurus and Monodelphis undoubtedly have different growth rates. These closely related didelphid marsupials (2) have similar life-spans of 2 to 3 years in the wild, yet Diphyurus reaches two to three times the adult size of Monodelphis (3). This accords with the observation that Diphyurus young remain attached to the teat longer and are weaned much later than Monodelphis young.

Do rates of maturation also differ? My observations on skeletal maturation in Monodelphis (4–5) agree closely with those of Smith (6) and van Nievelt, but we disagree on the timing of maturation events in Diphyurus. Their statements about Diphyurus are based on a study by Nesslinger (7), who examined only whole specimens that were cleared and stained for bone (alizarin). As histology shows, clearing and staining does not allow one to detect bone at its earliest stages in ontogeny. Nesslinger’s specimens consisted of only road-killed and wild-caught Diphyurus, so that chronological ages could only be approximated. More thorough studies on the embryology of Diphyurus (8–11) were based on a collection of several hundred specimens raised by the Wistar Institute in the 1930s. Histological sectioning of individuals of known ages indicates that, insofar as the skeletons of Monodelphis (4–6, 12) and Diphyurus (8–11) can be compared, they are virtually identical in timing of maturation.

For example, a synovial joint is present between the incus and malleus at birth in both Diphyurus (10) and Monodelphis (12). Ossification of the ectotympanic has begun by the middle of the second day in both species. In Diphyurus (10, p. 235) at 7 days the mandible has a definite temporomandibular articulation ... the mandibular condyle contains a larger condylar cartilage which has developed between the seventh and fifteenth day. It is rather large and is already undergoing some ossification ... just as in Monodelphis (5, 6, 12). Ossification of the malleus has begun in both Monodelphis (5) and Diphyurus (10) by the end of the second week. By the third week the incudo-malleolar joint is well formed and enclosed in a fibrous joint capsule in both species. In the fourth week, about the time of detachment, the incudo-stapedial joint becomes well formed and also enclosed in a fibrous joint capsule in both species. Over the remainder of ontogeny, the bones of the auditory chain in the two didelphids share similar chronologies. My examination of the surviving materials from the Wistar collection and other large North American skeletal collections of Diphyurus substantiates these observations (5); I can find no support for the statement that “any given event will occur 2 to 4 weeks later in Diphyurus than in Monodelphis.” Although didelphid species have different growth rates, their chronologies of maturation are closely comparable.

Last, the relation that I described between the brain and middle ear (4, 5) is one of relative growth, not timing of maturation. The relative size of the adult brain varies over more than an order of magnitude among different mammalian species, hence mammals must have widely varying rates of brain growth (13). But the small middle ear ossicles are far less variable in size, their growth ceasing early in ontogeny as a constraint of their function in high-frequency audition. Repositioning of the auditory chain occurs in the wake of continued cerebral growth. Didelphids are among the least encephalized mammals and offer the most generalized examples of this relationship. The patterns of variability among other species are invariably superimposed upon a more general pattern of differential growth of the brain and middle ear bones that is common to all mammals.

Timothy Rowe
Department of Geological Sciences and Vertebrate Paleontology Laboratory,
University of Texas,
Austin, Texas 78712, USA

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