the adult form (known as ontogeny) reveals details about the evolution and biology of a species that cannot be made by studying adult individuals alone. Series of fossils that chart the development of later tetrapods from larvae to adults[5] have provided a wealth of developmental data. However, the ontogenetic development of the earliest tetrapods has been poorly understood because such information is rare in the fossil record, and juvenile and adolescent stages had not been identified.

Sanchez and colleagues' study animal, *Acanthostega,* is one of the earliest known tetrapods, and lived about 365 million years ago during the Devonian period. The authors used synchrotron microtomography, a non-destructive way to generate 3D structural representations of the microstructure of fossil bone, and studied the long upper bone of *Acanthostega*'s forelimb, the humerus. Bone is a dynamic tissue, and studying its microstructure can reveal unique information about the physiology, growth and life history of vertebrates, because the internal structures provide indications of how fast an animal grew, how old an individual was and when growth ceased. Sanchez *et al.* investigated *Acanthostega* samples from a fossil assemblage site in which the individuals had all died together, probably in a drought following a catastrophic flood event. Although only a few humeri were available, they provide a glimpse into the growth patterns of this transitional species.

In tetrapods, the humerus initially forms as a cartilage precursor, with bone material being subsequently deposited in a process known as ossification. Surprisingly, Sanchez and colleagues' imaging data indicate that all of the specimens they investigated were still in the juvenile growth phase and had not reached sexual maturity. Even more surprisingly, *Acanthostega* seemingly reached almost its final size while retaining a cartilaginous humerus during an early-juvenile period that lasted several years (near final size was inferred when the bone microstructure showed that growth had slowed substantially). By contrast, ossification of the limb bones in modern tetrapods starts much earlier than in either *Acanthostega* or our fish predecessors. The finding that *Acanthostega* grew to almost final size and still had a cartilaginous humerus supports the hypothesis that the earliest tetrapods had a predominantly, if not an exclusively, aquatic lifestyle, because a cartilaginous humerus would probably have been unable to bear much weight. This indicates that limbs initially served a purpose on land other than locomotion.

However, the most compelling of Sanchez and colleagues' results lies in a clear disjunction between size and degree of ossification — some individuals reached the same degree of ossification in the long bones at a much smaller body size than others. Developmental plasticity, an organism's capacity to respond flexibly to different external cues throughout life, is thought to have an important role in evolution[6,7]. Studies of fossils and modern amphibians have elucidated the complex and fascinating connections between developmental plasticity and the responses of individuals to cues of population dynamics and environmental factors, including competition between juveniles, length of growth period, climatic factors and predation[8–11]. Sanchez *et al.* identified two size classes in their study (Fig. 1), although the small sample size limits interpretations with respect to possible drivers of plasticity. It is possible that there were more size classes, which may be revealed when further samples are available.

In *Acanthostega,* the decoupling of size and degree of ossification in a long juvenile stage could indicate that developmental plasticity, and possibly alternative life-history strategies, were already present in the earliest tetrapods. A high degree of developmental plasticity might have provided the means for our early ancestors to respond to changing intrinsic and environmental conditions, and could thereby have had a central role in the initial evolutionary success and subsequent diversification of tetrapods. ■

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IMMUNOLOGY

Cytotoxic T cells that escape exhaustion

T cells of the immune system mount antiviral responses, but if a response fails, a chronic viral infection can develop. It now seems that a T-cell subset in lymphoid immune tissues can control chronic infection. SEE LETTERS P.412 & P.417

CINDY S. MA & STUART G. TANGYE

Although T cells are known to fight chronic viral infections, the exact requirements for this process have been a mystery. Papers in this issue by Im *et al.*[1] (page 417) and He *et al.*[2] (page 412) and in *Nature Immunology* by Leong *et al.*[3] have identified a population of T cells that express the CXCR5 and CD8 surface proteins and may control these infections. These $CXCR5^+CD8^+$ T cells are located in immune tissues known as the secondary lymphoid system. The studies highlight the need to reassess the function of $CD8^+$ T-cell subsets in controlling chronic viral infections.

Cytotoxic $CD8^+$ T cells kill virus-infected cells and cancer cells[4], which they target by recognizing specific molecules called antigens. It is thought that $CD8^+$ T cells are absent from areas of secondary lymphoid tissues termed follicles, which are rich in immune B cells and are dedicated to generating antibodies. Follicles might therefore offer a location in which viruses can evade T-cell attack and so create a viral reservoir that could sustain a chronic infection[5]. The expression of CXCR5 enables a different type of T cell, known as a T follicular helper cell, to migrate into an area in these follicles called the B-cell zone[6–8]. T follicular helper cells also express the inhibitory receptor protein PD-1, and require a specific network of transcription factors for their development[6–8]. Although CXCR5 had been detected on a small fraction of $CD8^+$ T cells (less than 2%) in human blood and tonsils[9,10], the function of these cells was unclear. This has now been addressed by the current studies.

An experimental system for studying chronic viral infection is infection of mice with lymphocytic choriomeningitis virus (LCMV). LCMV infection causes a state known as immunological exhaustion, in which immunological attack on the infection is compromised because the $CD8^+$ T cells that target virus-infected cells show reduced production of immune cytokine signalling molecules, together with reduced proliferation and

cytotoxicity[11]. T-cell exhaustion occurs through various immune-cell regulatory pathways, including those that act through PD-1-mediated restraint of immune responses[11].

The authors of the three papers discovered that, during the chronic phase of LCMV infection, up to 30% of activated CD8$^+$ T cells expressed CXCR5 (Fig. 1). Im *et al.* and He and colleagues found that these cells were located exclusively in lymphoid tissues, and were involved in controlling the infection. The CXCR5$^+$CD8$^+$ T cells were found to share many features[6–8] and regulatory pathways[8,12,13] with T follicular helper cells, such as high expression of co-stimulatory receptor proteins and transcription factors. By contrast, the three studies found that the expression of immune inhibitory receptors associated with exhausted CD8$^+$ T cells[11] was reduced in CXCR5$^+$CD8$^+$ T cells compared with exhausted CD8$^+$ T cells that did not express CXCR5 (CXCR5$^-$CD8$^+$ T cells). PD-1 expression was found to be reduced (by Im *et al.* and He *et al.*) or at a similar level (Leong *et al.*) on CXCR5$^+$CD8$^+$ T cells compared with CXCR5$^-$CD8$^+$ T cells.

These exhausted CXCR5$^-$CD8$^+$ T cells were found throughout the 'red pulp' and T-cell-rich regions known as T-cell zones in the spleens of infected mice. However, all three studies found that CXCR5$^+$CD8$^+$ T cells homed to T-cell zones, and Im and colleagues and Leong *et al.* also found them in B-cell zones. He *et al.* and Leong *et al.* identified CXCR5$^+$CD8$^+$ T cells in humans infected with HIV or Epstein–Barr virus, both of which can establish chronic viral infection. Chronic viral infection in both humans and mice yields distinct responses of CD8$^+$ T cells depending on the cells' CXCR5 status. Studies in mice clearly showed that CXCR5$^-$CD8$^+$ T cells acquired characteristics of exhaustion and were distributed across both immune and non-immune tissues, whereas CXCR5$^+$CD8$^+$ T cells were confined to lymphoid tissues and showed features that were intermediate between those of T cells that had not been activated by antigen and those of exhausted T cells.

Im and colleagues and He *et al.* found that CXCR5$^+$CD8$^+$ T cells were better at controlling infection than CXCR5$^-$CD8$^+$ T cells. Transfer of LCMV-targeting CXCR5$^+$CD8$^+$ T cells into virus-infected mice resulted in a substantially lower viral load than when CXCR5$^-$CD8$^+$ T cells were transferred. He *et al.* found that the numbers of CXCR5$^+$CD8$^+$ T cells in people with HIV were inversely correlated with the viral load in the blood. The results suggest that CXCR5$^+$CD8$^+$ T cells are predominantly responsible for controlling chronic viral infections in humans and mice.

Intriguingly, gene-expression profiling of mouse CXCR5$^+$CD8$^+$ T cells by Im *et al.* revealed a molecular signature resembling that of blood stem cells. Consistent with this, these authors found that the cells proliferated extensively and yielded CXCR5$^-$CD8$^+$ T cells, indicating that CXCR5$^+$CD8$^+$ T cells are both stem-cell-like cells and precursors of exhausted T cells.

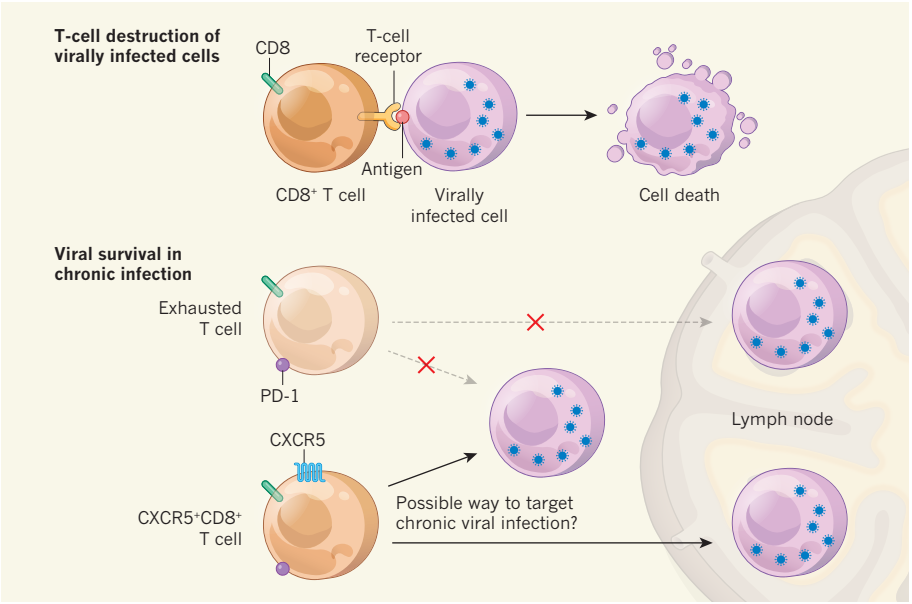

Figure 1 | A T-cell subset that targets chronic viral infection. When an immune T cell expressing CD8 protein (a CD8$^+$ T cell) is exposed to a specific viral antigen molecule that it recognizes through the T-cell receptor, the T cell becomes activated and can kill the virally infected cell presenting the antigen. In chronic infection, infected cells can escape T-cell-mediated destruction if T cells enter an 'exhausted' state in which proteins such as PD-1 inhibit T-cell activation. Viruses could also escape destruction in locations where T cells are absent, such as proposed T-cell-free areas including the B-cell zone of immune follicles in lymph nodes. Studies by Im *et al.*[1], He *et al.*[2] and Leong *et al.*[3] have identified a population of activated CD8$^+$ T cells that express the receptor protein CXCR5 and target chronic viral infection. These CXCR5$^+$CD8$^+$ T cells are not fully exhausted. The cells might be located in regions previously thought to be T-cell free, although the studies give conflicting reports of the location of the T cells.

Expression of PD-1 is a key determinant of whether T cells become exhausted. Leong and colleagues found that CXCR5$^+$CD8$^+$ and CXCR5$^-$CD8$^+$ T cells expressed comparable levels of PD-1, whereas Im *et al.* and He *et al.* found lower PD-1 levels in CXCR5$^+$CD8$^+$ T cells than in CXCR5$^-$CD8$^+$ T cells. Furthermore, Im *et al.* and He *et al.* found that the CXCR5$^+$CD8$^+$ T cells responded more robustly than CXCR5$^-$CD8$^+$ T cells to treatment with the antibody anti-PD-L1, which releases CD8$^+$ T cells from the suppressive effects of PD-1. This 'checkpoint inhibitor' treatment can curb immune-system impairment, and is yielding stunning results as immunotherapy for various cancers[14].

He *et al.* observed a synergistic antiviral effect when anti-PD-L1 treatment was combined with transfer of CXCR5$^+$CD8$^+$ T cells into infected mice. The effect was not seen with CXCR5$^-$CD8$^+$ T cells, suggesting that the antiviral effect of PD-1 inhibition is mediated exclusively by CXCR5$^+$CD8$^+$ T cells. Therefore, boosting the function of CXCR5$^+$CD8$^+$ T cells through PD-1 blockade is an attractive prospect for improving therapies for chronic viral infections, at least for infections that target lymphoid cells. If CXCR5$^+$CD8$^+$ T cells are responsible for viral eradication through an enhanced immunological response following PD-1 blockade, this raises questions about the function of CXCR5$^-$CD8$^+$ T cells and why these exhausted cells are retained during responses to chronic viral infections.

Although the three studies agreed on many aspects of the characterization of CXCR5$^+$CD8$^+$ T cells, a major difference was where these cells are located in the secondary lymphoid tissues. Im *et al.* convincingly demonstrated that the cells reside in the T-cell zone, but not in B-cell follicles, and that CXCR5$^-$CD8$^+$ T cells occur mainly in the splenic red pulp. These authors propose that CXCR5$^-$CD8$^+$ T cells kill virus-infected cells in splenic red pulp and non-lymphoid tissues, whereas CXCR5$^+$CD8$^+$ T cells recognize virus-infected cells in T-cell zones of lymphoid tissue. By contrast, He and colleagues and Leong *et al.* found that mouse CXCR5$^+$CD8$^+$ T cells occur with B cells in the B-cell zone of lymphoid tissue, and Leong and colleagues even found them in close association with HIV-infected cells in B-cell follicles of human lymph nodes.

Unlike the other studies, Leong *et al* found that CXCR5$^+$CD8$^+$ T cells expressed less-cytotoxic molecules than CXCR5$^-$CD8$^+$ T cells, and there was no difference in viral loads between mice that received LCMV-specific CD8$^+$ T cells from animals whose cells were able to express CXCR5 or from animals that lacked the gene enabling them to do so. Thus, in this experiment, whether or not the cells could express CXCR5 had no effect on the T cell's antiviral function. These

findings predict less efficacy of CXCR5$^+$CD8$^+$ T cells in viral control, especially in infection with HIV and Epstein–Barr virus, which respectively persist in follicles in immune T cells that express the CD4 protein and in B cells. Im and colleagues' finding of an absence of CXCR5$^+$CD8$^+$ T cells in follicles and Leong and colleagues' finding of reduced cytotoxicity of CXCR5$^+$CD8$^+$ T cells compared with CXCR5$^-$CD8$^+$ T cells are consistent with the idea that B-cell follicles provide a 'sanctuary' for HIV-infected T follicular helper cells[5]. The precise function and location of CXCR5$^+$CD8$^+$ T cells remain unresolved.

These studies provide insights into the spatio-temporal and dynamic nature of CD8$^+$ T-cell-mediated immunity against chronic infections. Parallels in the findings between humans and mice underscore the probable importance of CXCR5$^+$CD8$^+$ T cells in controlling protracted infections. Furthermore, the expansion and enhanced function of CXCR5$^+$CD8$^+$ T cells following PD-1 blockade render these cells a target for immune intervention when treating infectious disease. However, it is unknown whether these T cells are also generated in response to chronic viruses that infect non-lymphoid cells, such as hepatitis B or hepatitis C. It will be interesting to discover whether the cells infiltrate lymphoid and non-lymphoid tumours and so could also be targeted for cancer immunotherapy[14]. More studies of CXCR5$^+$CD8$^+$ T cells will be required before we can harness their full therapeutic potential. ■

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GENOMICS

Geography matters for *Arabidopsis*

A free database describes genome sequences, gene expression and molecular modifications to DNA for more than 1,000 *Arabidopsis thaliana* plants, providing valuable information on the complex history and current variation of this species.

OUTI SAVOLAINEN & MARTIN LASCOUX

The number of genomic resources is increasing rapidly, but large collections of high-quality, whole-genome sequences are available for only a few species, including humans[1] and fruit flies[2]. Such collections can help researchers to address both basic and applied genetic questions. Writing in *Cell*, the 1001 Genomes Consortium[3] and Kawakatsu *et al.*[4] describe an advanced genomic resource for the thale cress (*Arabidopsis thaliana*), molecular biology's most prominent plant model.

In the first study, the consortium presents whole-genome sequences of more than 1,300 genetically different individuals (accessions) from a worldwide collection (Fig. 1). In plants, such data sets have so far been generated only for cultivated species, such as rice[5] and tomatoes[6]. The *Arabidopsis* sequences are of high quality and easily accessible to users. The two papers jointly provide a good overview of all types of DNA-sequence variation.

Molecular modifications to DNA, such as the addition of methyl groups to the base cytosine, can influence gene expression and thereby alter physical and biological traits. In the second paper, Kawakatsu *et al.* improve on earlier studies (for example, ref. 7) by recording such epigenetic variants in about 1,000 accessions, which largely overlap with the sequenced accession set from the first paper.

What sets this work apart from most other collections of whole-genome sequence data is that seeds of all these accessions are freely available to the scientific community. *A. thaliana* is a predominantly selfing species — offspring receive two identical copies of each gene from the parent, whereas in outcrossing species, the copies from the mother and father can be different. The genome sequence of each accession of a selfing species is therefore maintained in subsequent generations. As such, researchers can obtain seeds whose genomes have already been fully characterized. Furthermore, the accessions also have data on gene expression and methylation status in some environments[4]. The groups have produced a tremendously valuable resource.

A crucial issue for this kind of large-scale study is the distribution of samples over

JAMES MANN/ABRC

Figure 1 | A catalogue of variation for *Arabidopsis thaliana*. Two studies[3,4] document genome sequences, gene expression and molecular modifications to DNA for more than 1,000 varieties of this plant species.