correlated with speed. In this clade, therefore, sperm size appears to have evolved as a secondary adaptation to sperm competition that is independent of the initial increase in sperm velocity.

The reasons for this macroevolutionary pattern remain unclear. One possibility is that an increment in speed may initially arise by morphological rearrangements of the sperm cell leading to higher thrust and reduced drag, for example through relative increase in the size of the midpiece, the mitochondrial engine of the sperm, and an optimization of the midpiece:flagellum ratio. These initial changes may have then set the scene for further selection on overall sperm length. Unfortunately, these sperm traits are difficult to measure in cichlid sperm and Fitzpatrick et al. [9] were unable to test these ideas. It is likely that resolving the mechanisms underpinning such evolutionary responses would require more information on the evolution of sperm morphology (for example, midpiece mass, flagellum length), and on the life-history trade-offs associated with sperm investment that male cichlids must have faced in their evolutionary past. But while much still needs to be

done to understand the evolutionary operation of sperm competition, this study represents a promising step ahead.

Darwin did not intuit sperm competition, so in a way this field represents a particular acid test of his evolutionary theory. He would have taken studies such as that by Fitzpatrick et al. [9] as a reassuring bicentenary present, confirming that his evolutionary theory is proving just as successful in explaining patterns of variation in sperm and ejaculate traits as it has been in explaining the very biological phenomena that inspired it.

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Natural Killer Cells: Remembrances of Things Past

Recent work has revealed that natural killer cells exhibit a form of memory, previously considered an exclusive property of adaptive immunity. While protective, natural killer cell memory is probably hazier and more fleeting than T cell memory.

David H. Raulet

The immune system is divided into adaptive and innate components [1]. The adaptive immune response — carried out by antibodies, T cells and B cells — is characterized by virtually unlimited diversity in specificity and exhibits long-term, antigen-specific memory responses when a pathogen is re-encountered years later. Innate immunity is carried out by many cell types, including granulocytes, macrophages, dendritic cells and natural killer (NK) cells. As innate immunity emerged as a field in recent years, textbooks defined the cardinal

features that distinguish it from adaptive immunity: innate immunity is characterized by recognition receptors of highly limited diversity, which target predictable features of pathogens and diseased cells, and is not accompanied by specific memory. Two recent studies [2,3] have now reported the surprising finding that NK cells do, in fact, exhibit a key feature of adaptive immunity: memory.

NK cells are lymphocytes, like T and B cells, and share many properties with CD8+ cytotoxic T cells [4]. They are nevertheless considered components of the innate immune system for several reasons: they exist in animals

that cannot produce T and B cells due to defects in enzymes necessary for rearranging T cell and B cell antigen receptor genes [5]; their recognition receptors do not undergo somatic diversification and are specific for predictable entities, such as ligands displayed on distressed cells or specific viral proteins [6]; they were not thought to confer long-term immunity to infections (memory).

In the adaptive immune system, memory is intimately tied to clonal diversity and clonal selection. For example, the frequency of CD8+ T cells that are specific for a viral antigen is perhaps 200 cells of the 10^8 CD8+ T cells in a naïve mouse, far too low to provide immediate protection [7] (Table 1). To provide any protection, clonal expansion of these specific T cells is crucial. Over a seven-day period of infection, these 200 cells expand at an astonishing rate, in some cases reaching a ceiling of more than 10^7 cells in the spleen alone, representing a 4–5 log increase [7].

After clearing the infection, most of the expanded T cells die, but a memory population of up to 10^6 cells per spleen persist, representing an almost 10^4-fold increase compared with a naïve animal [8]. Memory T cells localize in tissues where infections may occur and respond to antigen more rapidly and more robustly than naïve T cells. Hence, memory can be attributed to greater numbers of antigen-specific T cells as well as to more potent responses by each T cell. Considering the tiny number of antigen-specific T cells in a naïve animal, clonal expansion clearly plays a particularly important role in providing immunity to re-infection.

It has been appreciated for some time that NK cells display some features of adaptive immune cells [4]. For example, different NK cell clones display different sets of stimulatory and inhibitory receptors, creating a repertoire of specificities that is normally considered a feature of adaptive immunity; also, NK cells show specific self-tolerance that must be established in each individual. Upon exposure to an antigen that is recognized by only a subset of NK cells, this particular NK cell subset then 'clonally expands' over a period of several days [9]. And the possibility that NK cells have a form of memory was in fact suggested by several early reports, including a very early study of bone marrow graft rejection [10], as well as later studies showing that NK cells in naïve animals exist in a relatively inactive state and must be pre-activated to attain a sensitized state that provides more potent protection [11]. Sensitization could be induced by exposing NK cells *in vivo* to a sensitive tumor target cell [11] or to agonists that non-specifically activate NK cells, such as the innate immune stimulus poly(I:C) [12], or *in vitro* to cytokines such as interleukin-2 (IL-2) or IL-15 [13].

Though long recognized, these 'adaptive' features of NK cells seemed much less pronounced in NK cells than in T or B cells, and their significance had not been strongly emphasized. The NK cell repertoire is dramatically more limited than that of T cells or B cells, for example, and specific clonal expansion seemed to be much less robust. The sensitized state of activated NK cells was not thought to last long enough to justify the term 'memory'. In one of the new reports on NK cell memory,

Table 1. Comparison of NK cell and T cell responses and associated memory.

Cell type	Precursor frequency (cells per spleen)	Antigen-induced expansion (normal conditions)	Persistence of memory	Specificity of memory
CD8+ T cells	~200	10^4–10^5 fold	Years	High
NK cells (e.g. Ly49H+)	~2×10^6	3–10 fold (10^3-fold possible)	Weeks–months	Low?

Cooper et al. [3] now show that NK cells pre-activated with specific cytokines (IL-12 plus IL-18), and then transferred to recipient mice, persist for several weeks in a highly sensitized state compared with similarly transferred naïve NK cells. The duration of the sensitized state was striking (though the duration shown was much less than that of memory T cells) and would be expected to provide enhanced protection for a sustained period after an initial insult that activates NK cells. This form of memory was not specific, however, and did not require NK cell proliferation.

In the other new study, Sun et al. [2] document the potential for dramatic specific clonal expansion of NK cells in mice infected with murine cytomegalovirus (MCMV), and for persistence of expanded NK cells as memory cells. The activating NK cell receptor Ly49H, expressed by ~50% of NK cells, is specific for the viral m157 protein and is required for control of MCMV by NK cells. Ly49H+ NK cells specifically expand only 3–10-fold in infected mice [9], but Sun et al. [2] now show that up to 10^3-fold expansions could be achieved if the number of starting Ly49H+ NK cells was limited substantially by transferring small numbers of NK cells to mice with defective NK cells before infection [2] (Table 1). After some decay in numbers, expanded numbers of NK cells persisted for weeks after viral clearance and exhibited a sensitized phenotype. Transfer experiments indicated that these 'memory NK cells' were more protective against MCMV than were a comparable number of naïve NK cells. It is notable that the number of NK memory cells gradually decayed to approach the starting number, unlike memory CD8+ T cells, which stabilize at a greatly expanded number compared with the frequency in naïve mice. Although not tested, it would be of interest to test the specificity of the memory. Considering that NK cells are generally equipped with several types of receptors that can be used with some independence,

it is unlikely that the NK cell memory is highly specific.

It was impressive to observe such robust clonal expansion of Ly49H+ NK cells. It must be kept in mind, however, that the robust expansion was observed under artificial conditions in which the starting numbers of specific NK cells were purposefully limited. The normal starting frequency of Ly49H+ NK cells (approximately 2×10^6 cells per naïve spleen) is so very high compared with typical frequencies of antigen-specific T cells (~200 cells per naïve spleen) that clonal expansion is bound to be less important for mounting an effective response. Robust clonal expansion would presumably be of greater immunological benefit if rare NK cell specificities existed naturally in the pre-immune NK cell population. Such rare specificities have not yet been documented.

Specific NK cell memory was previously proposed by O'Leary et al. [14] and their results raise the possibility that rare NK cell specificities may exist. This study examined contact hypersensitivity responses, in which chemical agents that modify ('haptenate') proteins are painted on the skin and cause immune swelling reactions that were previously thought to be mediated exclusively by T cells; O'Leary et al. [14] showed that NK cells can also mediate this response. Surprisingly, contact hypersensitivity of NK cells is hapten-specific. Exposure of mice to one hapten sensitized them to respond strongly later when re-exposed to the same hapten, but not to a different hapten, and vice versa. Memory lasted for weeks, and could be transferred to a naïve animal with small numbers (10^5) of purified NK cells from a sensitized donor. While other interpretations are possible, the data raise the idea that hapten-specific NK cells were initially relatively rare in the naïve NK cell pool, and expanded in the memory pool. However, receptors expressed only rarely by NK cells have not been defined to date. The contact

hypersensitivity system, an *in vivo* reaction that involves poorly defined haptenated cell surface 'antigens', is not ideal for defining novel recognition receptors.

The new data mark an evolution from the view that NK cells respond *de novo* to each insult. The sustained sensitization of NK cells as a result of cytokines or infection at the least constitutes a form of hazy, fairly short-term memory, wherein a previous encounter ensures that NK cells will, for a period of weeks or months, lash out vigorously when exposed to the same or a different insult. The clonal expansion data and contact hypersensitivity data indicate that more specific NK cell memories exist as well, but a detailed understanding of how this works, and how important it is, awaits data that clarify whether novel NK cell receptors, or specificities, exist.

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Synaptic Transmission: Excitatory Autapses Find a Function?

An autapse is a synapse between a neuron and itself, a peculiar structure with an unclear function. A new study suggests that excitatory autapses contribute to a positive-feedback loop that maintains persistent electrical activity in neurons.

John M. Bekkers

Although the brain is complicated, we are told in undergraduate biology classes that the basic circuit element is simple: the axon of one neuron forms synapses on the dendrites of another neuron, and information is conveyed from cell to cell like the baton in a relay race. The reality is, of course, more complex. The brain has evolved other avenues of communication that seem to lack the sequential, neuron-to-neuron discipline of synaptic transmission. For example, neurotransmitter might spill out of the synaptic cleft and activate receptors on nearby neurons, including the neuron that released the transmitter [1,2]. Crosstalk could also occur between neurons and non-neuronal cells, such as glia [3].

Finallly, neurotransmission might occur at autapses.

An autapse is a self-synapse, a specialized structure in which a neuron forms a synaptic connection between its axon and its own dendrites [4,5]. Anatomical autapses are not uncommon in neural circuits, but their purpose has remained puzzling. Inhibitory autapses — those made by a neuron that releases an inhibitory neurotransmitter such as γ-amino-butyric acid (GABA) — seem to make sense because they could provide a self-stabilizing influence ('negative feedback') [6]. But what about excitatory autapses? Why should a neuron want to *de*stabilize itself by self-excitation in a positive-feedback loop? In a recent issue of *Current Biology*, Saada *et al.* [7] propose an answer to this question: that the

sustained activation of certain *Aplysia* neurons during feeding behavior may be aided by excitatory autapses. In this context, then, an excitatory autapse makes perfect sense: autaptic self-excitation tips the neuron into a hyperexcitable state that requires the persistent firing of action potentials.

Saada *et al.* [7] studied the buccal ganglia of *Aplysia*, focusing on B31/B32 neurons, motor neurons that drive muscles involved in grasping food during a repetitive feeding behavior in these animals [8]. When briefly stimulated in intact ganglia, B31/B32 neurons exhibit a prolonged depolarization and firing of action potentials (Figure 1), producing sustained muscle contraction. The authors wished to understand the mechanism of the persistent activity in B31/B32 cells.

These cells are embedded in a surprisingly complex circuit (Figure 1). They receive both fast and slow excitatory synaptic inputs mediated by release of acetylcholine from presynaptic neurons, the B63 cells. In addition, they are electrically connected to B63 cells via gap junctions. These connections make it difficult to establish with certainty the