Supplementary Methods:

A model of paAPC-T cell paracrine IL-2 delivery:

Fick's first law of diffusion relates concentration gradients and molecular fluxes. The concentration c_0 of released molecules at the surface of the particle of radius, a, is therefore related to the flux j_0 of molecules from the surface and their diffusion coefficient D (For an isolated single particle):

$$C = J_0 \frac{a}{D} \qquad \text{(Eqn.1)}$$

This relationship is valid whether the release rate is limited by the diffusion of molecules away from the surface or by the release of molecules from the particle. From figure 3A we estimated the flux J_0 of IL-2 molecules from a single particle. Since particles are on the average 8 um in diameter, their surface area is 200 um^2. They release about 500 pg of IL-2 per mg of particles during the first hour. Given that 1 mg of particles is about 1 million particles (measured by a particle counter), this corresponds to 0.033 pmol). Thus, the amount of IL-2 per particle is 3.3 x 10^{-20} mol and J_0 is 4.6 x 10^{-18} mol/(cm^2*s) which leads to a C_0 of 1.8 pM.

The IL-2 concentration field (C) is assumed to quasi-steady (QS), meaning it can vary only slowly with time. The paAPC and T cell are assumed to be spherical with radii of R_P=4 m and R_T=5 m, respectively. Under these conditions, the C-field is governed by the Laplace equation:

$$\nabla^2 C^* = 0 \qquad \text{(Eqn. 2)}$$

where C^* is the dimensionless concentration:

$$C^* = \frac{(C - C_\infty)}{(C_{Piso} - C_\infty)} \qquad \text{(Eqn. 3)}$$

C_{Piso} is defined as the surface concentration of IL-2 on the paAPC if it were isolated (far away) from the T cell. C_∞ is the concentration of IL-2 in culture, which is taken as zero.

It is convenient to use dimensionless quantities which are designated with an asterisk (*). For example, distances will be measured in units of R_P. Thus, the initial relative cell size (R_T^*) is R_T/R_P (=1.25) and the dimensionless separation (S^*) is S/R. The Laplacian operator in Eqn. (2) is normalized by R_P^2. The use of dimensionless quantities allows one calculation to be applied to many situations. For example, R_T^* =1.25 and S^* = 0.005 results can be applied to an 8m diameter paAPC interacting with a 10 m T cell separated by 20nm or a 4 m diameter paAPC interacting with a 5m T cell separated by 10nm, and so on.

The IL-2 molar flux is:

$$J = D_{ext}\nabla C = \frac{D_{ext}}{R_p}\left(C_{Piso} - C_\infty\right)\nabla^* = \frac{D_{ext}}{R_p}\left(C_{Piso} - C_\infty\right)J^* \qquad \text{(Eqn. 4)}$$

where ∇^* is the dimensionless gradient $\left(\frac{\nabla}{R_p}\right)$ and J^* is the dimensionless molar flux $\left(\nabla^* C^*\right)$.

$$J^* = \nabla^* C^* = \frac{JR_P}{D_{ext}\left(C_{Piso} - C_\infty\right)} \qquad \text{(Eqn. 5)}$$

The problem is not properly posed until the boundary conditions on the cell surfaces are specified. If the cells were perfect absorbers and emitters then these boundary conditions would simply be:

C^*= 1 on the paAPC and C^*= 0 on the T cell (Eqn. 6)

With these boundary conditions, the problem would be analogous to finding the electrostatic potential between two conducting spheres, one of unit size (R_P^* =1) raised to unit potential, and the other of relative size R_T^* =1.25 at ground and could be solved using, for example, the classical Method of Images (MOI) [44-47]. A perfect/perfect pair is of little interest because the IL-2 surface concentrations are known *a priori*. The dimensionless gradient between the surfaces at the synaptic point is nearly the same (1/S*) as that between two parallel plates separated by a distance S*. When S*= 0.005, corresponding to 20nm, the dimensionless gradient would be some 200 times greater than the gradient near an isolated paAPC. In light of the low internal diffusion coefficients typical of paAPCs, a paAPC is far from a "perfect source" and is incapable of supply IL-2 at such an elevated rate. Further, during the initial phase of paracrine delivery, when there are few if any receptors of high affinity on the T cell surface, the T cell is incapable of absorbing the IL-2 flux from a perfect source. Indeed, it is this initial phase of paracrine delivery that is the most interesting and will be the focus of what follows.

Instead of Eqn.(6), more realistic boundary conditions will be applied to the cells in this work. Suppose the local normal flux of IL-2 from the paAPC is dependent on the local surface concentration (C_P) according to:

$$J_p = \beta(C_0 - C_P) \qquad \text{(Eqn. 7)}$$

b plays the role of a first order kinetic rate constant for emission. Alternatively, b may be interpreted as a measure of the internal diffusive resistance in the paAPC. This may be modeled as an inner core with a uniform internal concentration of C_o and the IL-2 has to diffuse through a "membrane," of thickness L_{mem} and diffusion coefficient D_{mem}, to reach the paAPC surface. In this case, $b = D_{mem}/L_{mem}$. As opposed to diffusion through a true cell membrane, internal diffusion for paAPC may be modeled as diffusion through an

effective "membrane" that increases in thickness ($L_{mem,\,eff}^{*}$) with time. C_0 for a paAPC will decrease with time as the cell is depleted of IL-2. C_0 may be interpreted as the equilibrium or "saturation" surface concentration, at or above which the rate of emission is zero. C_P, in general, is a function of position along the paAPC surface. Clearly J_P will be greatest where C_P is smallest and have a maximum rate of b C_o when the surface is free of IL-2 ($C_P = 0$). When the surface is saturated, $C_P = C_o$, the local flux is zero.

Equation (7) may be written in dimensionless form as:

$$J_P^* = \beta^* (C_0^* - C_P^*) \qquad \text{(Eqn. 8)}$$

where

$$\beta^* = \frac{D_{mem} R_P}{D_{ext} L_{mem}} = \frac{D_{mem}}{D_{ext} L_{mem,eff}^*} \qquad \text{(Eqn. 9)}$$

In this case, when the paAPC is far from the Tcell, it follows from the definitions of the dimensionless quantities that $J_P^* = 1$ and

$$C_0^* = 1 + \left(\frac{1}{\beta^*}\right) \qquad \text{(Eqn. 10)}$$

A small b^* then means significant diffusional resistance and a large C_0^*. A large b^* implies little internal resistance and $C_P^* \sim C_0^* \sim 1$. Significant internal resistance, however, is expected. D_{int} in a paAPC is typically several orders of magnitude lower than D_{ext} resulting in extremely small b^* values during most of the paAPC's release lifetime. Fortunately, the dimensionless calculations are not sensitive to the precise value of b^* provided $b^* << 1$. For illustrative purposes, a b^* of 10^{-4} will be used here. C_0^*, therefore, will be orders of magnitude higher than C_P^*. A large C_0^* does not imply an absurdly high internal concentration, but rather an extremely low value of C_{Piso}.

Substituting Eqn.(10) into Eqn.(8) yields an alternative form for the dimensionless normal flux that does not explicitly depend on C_0^*:

$$J_P^* = 1 + \beta^* (1 - C_P^*) \qquad \text{(Eqn. 11)}$$

From this alternative form, it is clear from the above that as $b^* \rightarrow 0$, $C_P^* \rightarrow 0$ and $J_P^* \rightarrow 1$, the same value as for an isolated particle. This small b^* limit, will be referred to as the "constant flux limit." In this limit, the rate-determining step is internal diffusion and the rate of emission becomes remarkably insensitive to interactions. With $D_{int} << D_{ext}$, paAPCs, except when they are "fresh," will fall into this constant source limit over most of their release lifetimes. The reason for this is the driving force for internal diffusion is $C_o^* - C_P^*$, and since $C_o^* >> 1$ (C_{Piso}^*), any slight changes in C_P^* due to interactions does not appreciable change the internal diffusion rate. While J_P^* may be nearly constant and the same as an isolated cell, C_P^* will tend to vary over the surface and will not necessarily

have the isolated particle C_{Piso}^* value of unity. In the constant source flux limit, interactions affect the surface concentrations but not the rate of emission.

Eqn. (8), therefore, is the boundary condition on the surface of the paAPC. The Tcell, on the other hand, is assumed to be non-absorbing, so the normal surface flux of IL-2 on the T cell surface is zero:

$$J_T^* = 0 \qquad \text{(Eqn. 12)}$$

The problem now is to solve the Laplace equation, (Eqn. 2), subject to the boundary conditions represented by Eqn. (8) on the paAPC and (Eqn.12) on the T cell. While b^* can be estimated *apriori*, the local surface concentrations (C_P^* and C_T^*) cannot. Further, these concentrations will not be uniform and will vary point to point along the cell surfaces.

As in Refs (19,20), boundary collocation method is used to solve this problem. The field near a point or ring singularity satisfies the Laplace equation. Since the Laplace equation is linear, a solution can be crafted by superimposing the fields of a series of singularities of suitable strength so as to satisfy the boundary conditions. In a boundary collocation method, the boundary conditions are satisfied at N discrete points. While with using this method the boundary conditions are not satisfied everywhere, by choosing N to be sufficiently large, a reasonable solution may be obtained.

The concentration at any point (x) between the cells can then be expressed as series:

$$C^*(x) = \sum q_i f_i(x) \qquad \text{(Eqn. 13)}$$

where q_i is the strength of the ith source and f_i is the contribution to the field from that source when q_i were equal to unity.

Since the problem is axially symmetric, it is convenient to use ring singularities. The field near a ring singularity of unit strength is:

$$f_i(x) = \frac{2K(z)}{\pi w^{1/2}} \qquad \text{(Eqn. 14)}$$

$$w = r^2 + R_c^2 + a^2 + 2rR_c$$

$$z = \left(\frac{4rR_c}{w}\right)^{1/2}$$

Where K(z) is the complete elliptical integral of the first kind, R_c is the radius of the ring, and a and r are the axial and radial distances from the center of the ring to the $\underline{x}$ point.

The N equations for the N unknown values of q_i are found by satisfying the boundary conditions at N discrete points.

The jth collocation point is on the paAPC:

$$\sum_{\substack{i=1 \\ i \neq j}}^{N} q_i (g_{ij} - f_{ij}) = -\beta^* C_0^* \qquad \text{(Eqn. 15)}$$

where f_{ij} is contribution of the ith source to the field at the jth collocation point. g_{ij} is the contribution of the gradient of f_{ij} normal to the surface at the jth collocation point.

If the jth collocation point is on the T cell then:

$$\sum_{\substack{i=1 \\ i \neq j}}^{N} q_i (g_{ij}) = 0 \qquad \text{(Eqn. 16)}$$

Eqns.(15) and (16) represent N linear equations which can be solved for the N unknown q_i. Knowing the q_i, the surface concentrations at the N points can be calculated from Eqn.(13). Once the q_i are known, the field anywhere on the surface of or between the cells can be found from Eqn.(13). Knowing the surface concentrations, the local normal fluxes follow from Eqns.(8) and (12).

The ring singularities were arranged on a “singularity sphere” that is roughly 95% of the radius inside a given cell. The radius of the singularity sphere is an important computational variable and is found by trial and error. If the radius is too large, then large variations between the collocation points may occur. If the radius is too small, then round-off errors become troublesome. Using a 4 degree (dq_s) singularity separation, the maximum error in C^* at the midpoint between two collocation points was found to be less than 0.1% in most cases.

References:

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