

OKT3

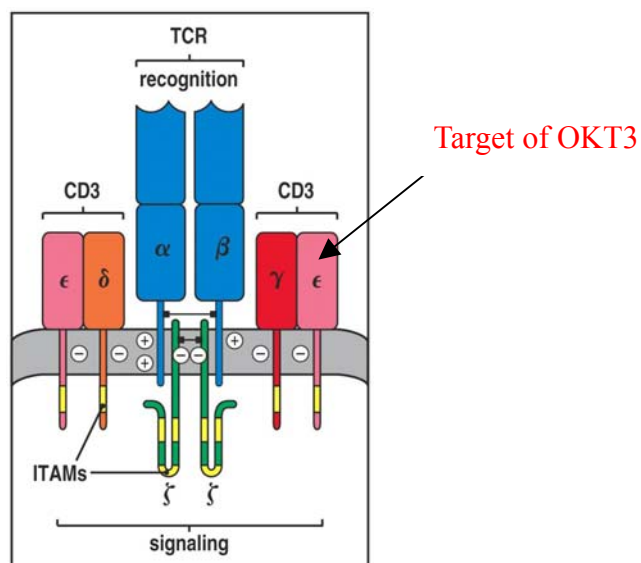
~ The first mouse monoclonal antibody

used in clinical practice in the field of transplantation ~

As everybody knows, OKT3 is the first mouse monoclonal antibody produced for the treatment of acute transplant rejection. It is remarkable that this clinical use of OKT3 occurred long before the molecular complexities of the CD3–T-cell receptor (TCR) complex were discovered and the key signaling role of CD3 was unraveled. Moreover, though more than 20 years have passed since it was first administered to a patient, it still has large market potential now. Therefore, I choose this topic, organizing the review papers I read, and finally propose some of my discussions.

Background knowledge about OKT3

The development of OKT3 (Ortho Biotech) — a monoclonal IgG2a antibody, produced in 1979 by P.Kung and G. Goldstein, which binds the ϵ component of the CD3 signal-transduction complex— is an important advance for induction and anti-rejection therapy. It functions by inducing selective modulation and inactivation of T cells.



Mechanism:

It binds to ϵ chain of CD3 signal transduction complex, strongly inhibits the proliferation of alloreactive lymphocytes and the generation of allospecific cytotoxic effectors. Therefore, it can more specifically suppress the immune response in organ transplantation than Cytotoxic drugs such as azathioprine or cyclosporine A.

Illustrating the unique potency of OKT3, treatment with OKT3 alone, in the absence of any other associated treatment, could prevent rejection and sustain the normal function of a mismatched human renal allograft while significant levels of the monoclonal antibody were present in the circulation.

T cell depletion owing to Fc receptor?

Within 10 to 20 minutes of a first injection of OKT3, CD3+ T cells disappear from the peripheral. Initially, this was interpreted wrongly as owing to the large-scale depletion of T cell.

However, data showed that CD3-specific antibodies that bind Fc receptors (FcRs), such as OKT3, induce only a partial depletion (40–50%) of the total number of CD3+ lymphocytes in peripheral blood and the various lymphoid organs.

The divalent antigen-binding portion also plays parts in T cell depletion

The T-cell-depleting capacity of CD3-specific monoclonal antibodies depends not only on the effector functions of the Fc portion of the antibody — complement-mediated depletion and antibody dependent cellular cytotoxicity (ADCC) — but also on the divalent antigen-binding portion.

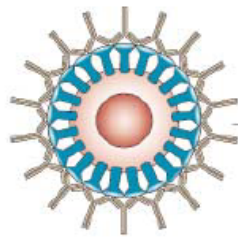
Long term effects – internalization of TCR complex

CD3+ cells that do not disappear physically still completely lose expression of CD3 and consequently also of the linked TCR. This phenomenon is the result of CD3-specific antibody mediated internalization, and catabolism or recycling of the target CD3–TCR molecular complex.

Mode of action of therapeutic monoclonal antibodies

Therapeutic T cell specific monoclonal antibodies act through four mechanisms that are not mutually exclusive::

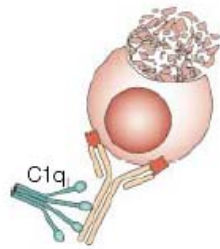
1. Coating



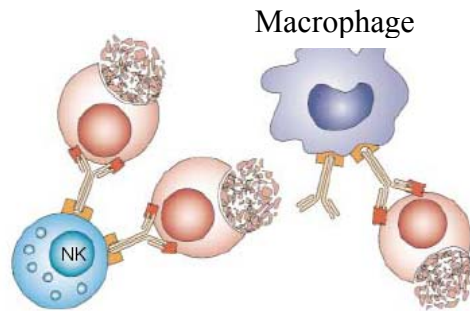
Prevents cell-cell interactions
Delivers negative signals

2. Depletion

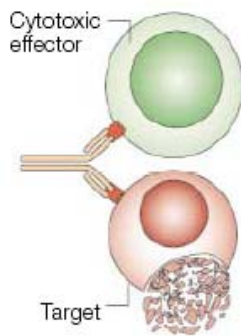
a). complement mediated



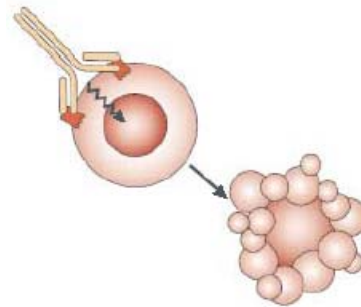
b). opsonization/ADCC



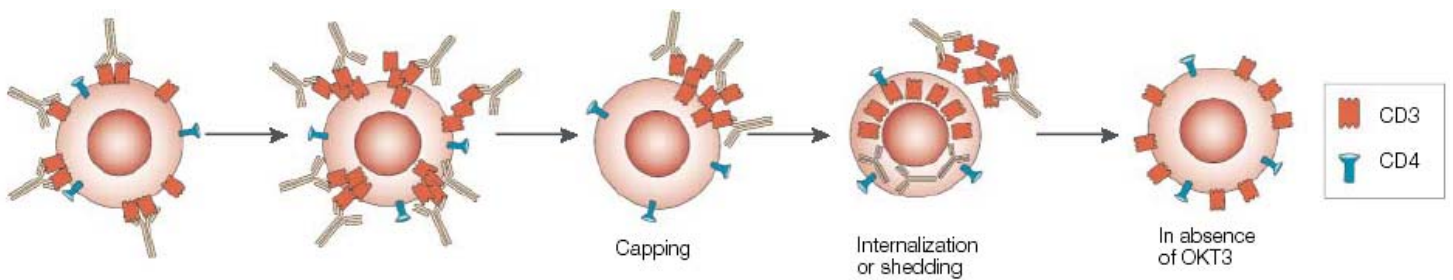
c). redirected cell lysis



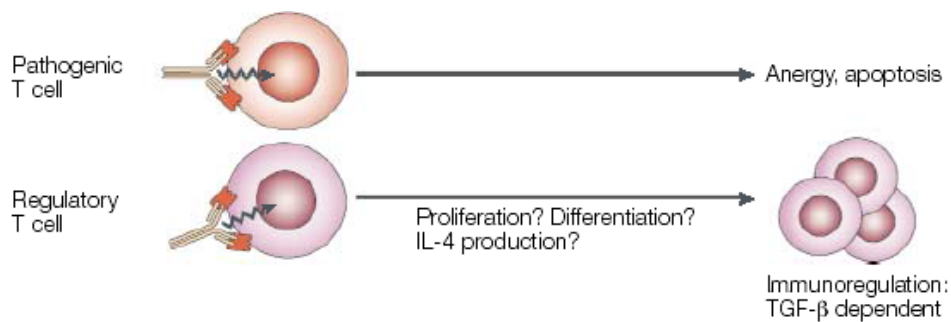
d). Apoptosis



3. TCR down-modulation



4. cell signaling



TCR down-modulation and T-cell signaling seem to be the main mechanisms that are used by CD3-specific antibodies. T-cell depletion is also observed, but to a much lesser extent, in particular with non-Fc receptor (FcR)-binding CD3-specific antibodies. Cell coating is the main mode of action of monoclonal antibodies specific for CD4. Not only does it prevent cell–cell interactions, but it also elicits ‘negative signals’, the molecular basis of which has not been elucidated fully.

Side effects

The clinical use of OKT3 was hampered by serious side effects linked to its immunogenic and mitogenic potentials, which limited its more widespread use in transplantation.

An important side effect, which precluded the use of therapy with OKT3 alone, was the massive OKT3-specific humoral response that developed rapidly (by day 5 to 7 of treatment), which neutralized and cleared the antibody and precipitated acute rejection.

1. Immunogenicity: human anti-mouse antibody response

The occurrence of an anti-globulin response (mainly IgM and IgG) to the xenogeneic protein, despite the profound antibody-induced immunosuppression, was one of the main drawbacks in early trials of OKT3, because it promoted the rapid clearance and neutralization of OKT3.

Thus, with this immunogenicity, why OKT3 can still be marketed?

~~ Owing to Specificity restriction ~~

Immunochemical analysis of purified OKT3-specific IgM and IgG antibodies indicated that the response is remarkably restricted, consisting exclusively of antibodies specific for isotypic (IgG2a-specific antibodies; OKT3 is a mouse IgG2a) and idiotypic determinants.

Moreover, the anti-globulin response recruits only a few specific B-cell clones. Therefore, its neutralizing potential is dependent not only on the titers of the antibodies produced but also, and perhaps more importantly, on their fine specificities. This might explain why, in contrast to sensitization to polyclonal antisera, sensitization to OKT3 never led to clinically overt immune-complex disease (serum sickness) — the amount of immune complexes formed was probably insufficient to allow sustained tissue deposition.

2. Mitogenicity: cytokine release

Most of the monoclonal antibodies that were raised initially against human, mouse or monkey CD3 were mitogenic. They induced T-cell proliferation and large-scale release of cytokines, including many T-cell derived pro-inflammatory cytokines such as TNF.

The mitogenic capacity of CD3-specific antibodies is, however, monocyte/macrophage dependent, and it involves the production of interleukin-6 (IL-6) and IL-1 β by these cells. IL-1 β was detected only in association with cells (probably the transmembrane form), and never in the circulation.

Although it is transient, this cytokine release leads to a 'flu-like' syndrome, which is characterized by fever, chills, headaches, nausea, vomiting, diarrhoea, respiratory distress, septic meningitis and hypotension. It is mainly the combination of TNF, IL-6 and interferon- γ (IFN- γ) that causes the typical syndrome

There are now some approaches to reduce the immunogenicity and mitogenicity:

1. Removing Fc portion

The mitogenicity of CD3-specific antibodies correlates with the capacity of the Fc portion to interact with FcRs that are present on phagocytes and natural killer cells. So, CD3-specific (Fab)₂ fragments, which lack the Fc portion, are not mitogenic *in vitro*.

2. Choosing low affinity mouse antibody isotype

The proliferative response differs according to the affinity of the mouse antibody isotype for human monocytes Fc γ RI, Fc γ RII and Fc γ RIII (IgG2a > IgG1 > IgG2b > IgA). This fits with clinical observations that mouse CD3-specific antibodies of the IgA isotype did not promote large-scale cytokine release and were well tolerated.

3. Administering single high dose of corticosteroid at least one hour before infection of OKT3.

The use of OKT3 in association with conventional immunosuppressants helped to decrease the antiglobulin response. Adding corticosteroids and azathioprine decreased the frequency of sensitization to OKT3 from 95% to 40%. Addition of cyclosporin further reduced the frequency of sensitization to 20%, and, in most cases, the antibodies that were produced did not affect the efficacy of OKT3 because they appeared only by the end of treatment with OKT3

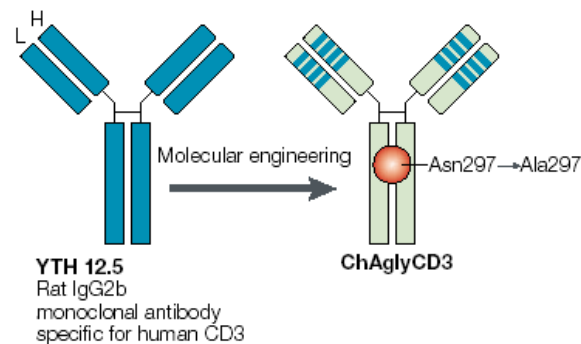
Genetically engineered CD3-specific antibodies

There is now compelling evidence that humanized antibodies, obtained by molecular engineering, are less immunogenic than their non-humanized counterparts. Through genetic engineering, recombinant antibodies specific for human CD3 have been produced that contain the parental rodent antibody hypervariable regions (which determine antigen specificity) grafted within a mutated non-FcR-binding human IgG1 heavy- and light-chain immunoglobulin.

Two of these antibodies are being tested at present in Phase I and II trials in autoimmunity and transplantation:

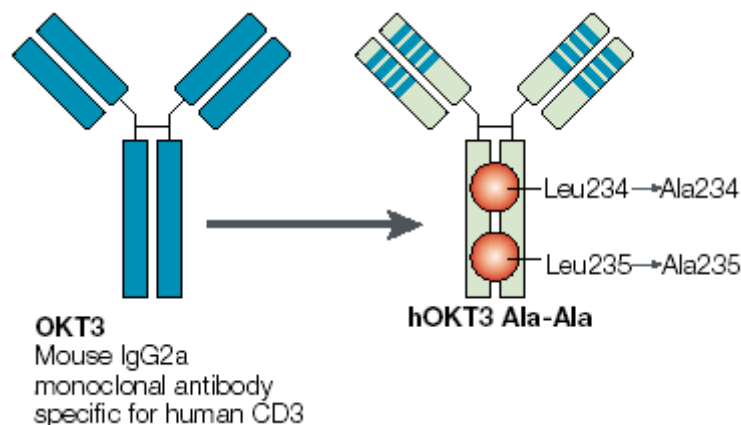
1. ChAglyCD3

It's a humanized non-mitogenic version of the rat YTH 12.5 antibody. It consists of a $\gamma 1$ constant region that lacks the CH2-domain glycosylation site. Previous studies have shown that aglycosylated antibodies cannot bind FcRs or activate complement. ChAglyCD3 fails to induce human T-cell proliferation and has a reduced ability to direct T cells to kill cells bearing human FcRs.



2. huOKT3 $\gamma 1$ Ala-Ala

It's a humanized non-mitogenic version of OKT3. It did not promote significant cytokine release when injected into severe combined immunodeficient (SCID) mice reconstituted with normal human splenocytes.



CD3 specific antibodies induce tolerance

Data from three different experimental models provide strong evidence in favor of the capacity of CD3- specific antibodies to induce operational tolerance:

During the tolerance-induction phase (the first 12 days after transplantation), alloreactive cells were not eliminated and a 'non destructive' infiltrate, composed mainly of CD4+ cells was present in the heart allografts of CD3-specific antibody-treated recipients. However, increased levels of mRNA encoding IL-4 and IL-5 were present in the spleen. This **T HELPER 2 (TH2) polarization** is in keeping with in vitro studies showing the preferential induction of TH2-cytokine production by activated T-cell lines incubated with CD3-specific antibodies, particularly non-FcR-binding antibodies.

More recently, studies from my laboratory have indicated that **regulatory T cells** are present in CD3-specific antibody-treated tolerant mice. These cells are detected by their ability to inhibit the transfer of disease by diabetogenic lymphocytes (from the spleen of overtly diabetic NOD mice). These 'protective' T cells express L-selectin (CD62L) and are found mostly in the CD4+CD25+ lymphocyte compartment.

In terms of mechanisms, it seems that CD3-specific antibodies are representatives of a new category of immunotherapeutic agents that induce tolerance through their capacity to induce immunoregulatory T cells. It is remarkable that these regulatory cells become concentrated in the close vicinity of the target organ.

Conclusion

Today, CD3-specific antibodies are representative of a new category of immunotherapeutic agents, which might provide a cure for established autoimmunity and allow the long-term survival of organ allografts.

However, there are still problems remained to be solved:

1. human anti-mouse antibody response
2. acute toxicity and systemic cytokine release
3. induced a large amount of procoagulant activity (PCA) in human peripheral blood mononuclear cells (PBM)

Two recent developments have allowed the increased clinical use of CD3-specific antibodies:

1. The production of **HUMANIZED ANTIBODIES** specific for CD3 with mutated Fc regions (which are safe): **ChAglyCD3** and **huOKT3 γ 1 Ala-Ala**
2. The demonstration that short-term treatment with CD3-specific monoclonal antibodies can elicit **OPERATIONAL TOLERANCE** in transplantation and autoimmunity.
3. The PCA-inducing capability in OKT3 MoAb was abolished by absorption with T lymphocytes or Sepharose-conjugated antibody to mouse IgG

Discussion

The murine monoclonal antibodies (mAb) OKT3 with specificity for the human CD3 complex exerts two seemingly contradictory effects, namely a transient activation of T cells occurring shortly after the stimulation and a persistent immunosuppression which has given rise to a number of clinical applications to prevent organ rejection.

As mentioned above, the therapeutic action of anti-CD3 monoclonal antibody involves T helper 2 cell polarization and induction of regulatory CD4⁺IL-10⁺ T cells. Therefore, anti-CD3 may have potential use in other clinical field such as **autoimmune disease** mediated by T cells, for example, type I Diabetes.

So, since anti-CD3 mAb can be used for treating some T cell mediated autoimmune disease, I am curious about if it can also be used for controlling **type IV hypersensitivity** which is also mediated by T cells?

In addition, the cytokines produced in response to OKT3 can be classified into two categories: One involves immune response: IL-4, IL-2, TNF- α ; the other involves hematopoiesis: GM-CSF, IL-3, and IL-6. We may apply these cytokines for **tumor rejection** and **stem cell modification**; however, we must be very careful in deciding the most suitable dose which induces a potent T cell activation without significant T cell depletion from the spleen.

Reference

1. NATURE REVIEWS | IMMUNOLOGY VOLUME 3 | FEBRUARY 2003 | 123-132 **CD3-SPECIFIC ANTIBODY-INDUCED ACTIVE TOLERANCE: FROM BENCH TO BEDSIDE**
2. The Journal of Immunology, Vol 139, Issue 5 1617-1623, Copyright © 1987 by American Association of Immunologists **Induction of monocyte procoagulant activity with OKT3 antibody** (only abstract)
3. *Ann. N.Y. Acad. Sci.* **1037**: 1 – 9 (2004). doi: 10.1196/annals.1337.001 **Use of Anti-CD3 Monoclonal Antibody to Induce Immune Regulation in Type 1 Diabetes** (only abstract)
4. The Journal of Immunology, Vol 137, Issue 3 830-838, Copyright © 1986 by American Association of Immunologists **Restriction of the human in vivo immune response against the mouse monoclonal antibody OKT3** (only abstract)